

1990

# Genotypic and Phenotypic Adaptations of the Southern Oyster Drills, *Stramonita Canaliculata* and *S. Haemastoma Floridana* to Salinity.

Li-lian Liu

*Louisiana State University and Agricultural & Mechanical College*

Follow this and additional works at: [https://digitalcommons.lsu.edu/gradschool\\_disstheses](https://digitalcommons.lsu.edu/gradschool_disstheses)

---

## Recommended Citation

Liu, Li-lian, "Genotypic and Phenotypic Adaptations of the Southern Oyster Drills, *Stramonita Canaliculata* and *S. Haemastoma Floridana* to Salinity." (1990). *LSU Historical Dissertations and Theses*. 4932.  
[https://digitalcommons.lsu.edu/gradschool\\_disstheses/4932](https://digitalcommons.lsu.edu/gradschool_disstheses/4932)

This Dissertation is brought to you for free and open access by the Graduate School at LSU Digital Commons. It has been accepted for inclusion in LSU Historical Dissertations and Theses by an authorized administrator of LSU Digital Commons. For more information, please contact [gradetd@lsu.edu](mailto:gradetd@lsu.edu).

## INFORMATION TO USERS

The most advanced technology has been used to photograph and reproduce this manuscript from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

**The quality of this reproduction is dependent upon the quality of the copy submitted.** Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

# U·M·I

University Microfilms International  
A Bell & Howell Information Company  
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA  
313/761-4700 800/521-0600



**Order Number 9104151**

**Genotypic and phenotypic adaptations of the southern oyster  
drills, *Stramonita canaliculata* and *S. haemastoma floridana* to salinity**

**Liu, Li-Lian, Ph.D.**

**The Louisiana State University and Agricultural and Mechanical Col., 1990**

**U·M·I**

**300 N. Zeeb Rd.  
Ann Arbor, MI 48106**



GENOTYPIC AND PHENOTYPIC ADAPTATIONS OF THE SOUTHERN OYSTER DRILLS,  
STRAMONITA CANALICULATA AND S. HAEMASTOMA  
FLORIDANA TO SALINITY

A Dissertation

Submitted to the Graduate Faculty of the  
Louisiana State University  
Agricultural and Mechanical College  
in partial fulfillment of the  
requirements for the degree of  
Doctor of Philosophy

in

The Department of Zoology and Physiology

by

Li-Lian Liu

B.S., National Taiwan Normal University, Taiwan, 1981

M.S., National Sun Yat-Sen University, Taiwan, 1983

May, 1990

#### ACKNOWLEDGEMENTS

I would like to express my appreciation to my major professor, Dr. W.B. Stickle for his support and guidance throughout my graduate studies. This work would not have been possible without the assistance of Dr. D.W. Foltz, who has spent many hours teaching me the techniques of horizontal starch gel electrophoresis and data analysis. Also, I would like to thank Drs. S.G. Bartlett, K.M. Brown, T.H. Dietz, J.W. Fleege, E. Gnaiger, and J.F. Siebenaller for their help and encouragement. Mr. P. Rutledge, Mr. W. Delaune, Ms. M. Williams, Mr. C. Hopkins, Mr. R. Bouchard Jr. and many other people who shared their knowledge and offered their help to me in many different ways. I also wish to acknowledge the National Science Council of Taiwan for the two-year (1986-1988) scholarship which supported my study in United States.

# TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	ii
TABLE OF CONTENTS.....	iii
LIST OF TABLES.....	iv
LIST OF FIGURES.....	v
ABSTRACT.....	1
INTRODUCTION.....	3
CHAPTER 1. THE SYSTEMATICS OF OYSTER DRILLS, <u>STRAMONITA</u> " <u>THAIS</u> " IN THE SOUTHERN UNITED STATES.....	5
ABSTRACT.....	5
INTRODUCTION.....	5
MATERIALS AND METHODS.....	7
RESULTS.....	17
DISCUSSION.....	29
ACKNOWLEDGEMENTS.....	38
LITERATURE CITED.....	39
APPENDIX.....	45
CHAPTER 2. ADAPTATION TO SALINITY IN <u>STRAMONITA</u> " <u>THAIS</u> " <u>CANALICULATA</u> AND <u>S. HAEMASTOMA FLORIDANA</u> : PATTERNS OF PHENOTYPIC AND GENOTYPIC VARIATIONS.....	52
ABSTRACT.....	52
INTRODUCTION.....	52
MATERIALS AND METHODS.....	57
RESULTS.....	63
DISCUSSION.....	67
ACKNOWLEDGEMENTS.....	73
LITERATURE CITED.....	74
APPENDIX.....	80
CHAPTER 3. NORMOXIC AND ANOXIC ENERGY METABOLISM OF THE SOUTHERN OYSTER DRILL <u>STRAMONITA CANALICULATA</u> DURING SALINITY ACCLIMATION. A DIRECT CALORIMETRIC STUDY .....	83
ABSTRACT .....	83
INTRODUCTION.....	83
MATERIALS AND METHODS.....	85
RESULTS.....	87
DISCUSSION.....	98
ACKNOWLEDGEMENTS.....	102
LITERATURE CITED.....	103
SUMMARY.....	107
LITERATURE CITED.....	110
COPYRIGHT RELEASE.....	114
VITA.....	116



## LIST OF TABLES

Table 1.1	Collection sites of <u>Stramonita</u> .....	10
Table 1.2	Enzymes used for electrophoresis.....	16
Table 1.3	Shell measurements of six populations of <u>Stramonita</u> .....	18
Table 1.4	Radular variations of <u>Stramonita</u> .....	21
Table 1.5	Shell measurements of three <u>Stramonita</u> species.....	30
Table 2.1	<u>Stramonita</u> species collection sites.....	60
Table 2.2	Summary of the variance for Wright's F-statistics.....	62
Table 2.3	28-day LC-50 values of <u>Stramonita</u> .....	64
Table 2.4	Wright's hierarchical F-statistics of <u>Stramonita</u> .....	68
Table 2.5	Summary of genetic variations in <u>Stramonita</u> .....	69
Table 3.1	<u>Stamonita canaliculata</u> mean steady-state heat flux.....	88

## LIST OF FIGURES

Fig. 1.1	Collection sites of <u>Stramonita</u> .....	9
Fig. 1.2	Representative shells of <u>Stramonita</u> .....	12
Fig. 1.3	SEM of the radula of <u>Stramonita</u> .....	20
Fig. 1.4	First principal component values of <u>Stramonita</u> .....	25
Fig. 1.5	An UPGMA cluster analysis of <u>Stramonita</u> .....	28
Fig. 1.6	World distribution of four subspecies of <u>S. haemastoma</u> ...	34
Fig. 2.1	Summary of metabolic pathways.....	56
Fig. 2.2	Collection sites of <u>Stramonita</u> .....	59
Fig. 2.3	The 28-day LC-50 of five populations of <u>Stramonita</u> .....	66
Fig. 3.1	Heat flux of <u>S. canaliculata</u> during salinity transfer from 10 to 30 ‰ under normoxia.....	90
Fig. 3.2	Heat flux of <u>S. canaliculata</u> during salinity transfer from 30 to 10 ‰ under normoxia.....	92
Fig. 3.1	Heat flux of <u>S. canaliculata</u> during salinity transfer from 10 to 30 ‰ under anoxia.....	94
Fig. 3.1	Heat flux of <u>S. canaliculata</u> during salinity transfer from 30 to 10 ‰ under anoxia.....	96

## ABSTRACT

Environmental and genetic effects on salinity adaptation in the southern oyster drill were estimated by their salinity-related genotypic (i.e. determined by electrophoresis) and phenotypic (i.e. determined by salinity tolerance and microcalorimetry) variations. The systematics of Stramonita haemastoma were studied using allozyme, radular and shell characteristics to exclude taxonomic differences which might have obscured variations in salinity adaptation. Radular and shell features are not reliable as systematic characteristics. S. h. canaliculata and S. h. floridana are separable using allozyme characteristics. Nei's genetic distance ( $D=0.28$ ) and the fixed allelic differences in Fum, LeuAla, Mdh, and 6-Pgdh indicate that the differences between S. h. canaliculata and S. h. floridana are on the species level and S. h. canaliculata should be named S. canaliculata.

28-day high-salinity LC-50 is lower in four populations of Stramonita canaliculata (44.4 to 51.5 ‰ S) than in the population of S. haemastoma floridana (54.8 ‰ S). In low salinity, S. canaliculata (3.5 to 7.1 ‰ S) is more tolerant than S. h. floridana (7.3 ‰ S). S. canaliculata from medium to high salinity areas exhibit a higher high-salinity LC-50 and shorter adaptation period than populations from low to medium salinity. Low-salinity LC-50 exhibit the opposite trend. Little allelic variation of loci has been found among populations in either species.

Heat flux of Stramonita canaliculata was depressed less with a shorter recovery period in snails transferred from normoxic 10 to 30 ‰ S than snails transferred from 30 to 10 ‰ S. Snails transferred to hyperosmotic conditions expended more energy than snails transferred to hypoosmotic conditions under anoxia. Phenotypic variations among individuals exist as indicated by the heat flux variability in these experiments.

Salinity adaptation in the southern oyster drills is primarily due

to environmental effects as observed in the phenotypic variations in salinity tolerance and heat flux with little genetic variation among populations. Their protein-oriented metabolism and avoidance behavior to rapid salinity changes may explain the insignificant genotype specific adaptation to salinity.

## Introduction

Any response on the part of an organism that favors survival in the face of changes in the external environment is called adaptation and is determined by genetic, environmental, and developmental factors (Prosser, 1986). According to Prosser (1986), a phenotype is completely described when both the genotype and the environment have been specified after an organism has passed through essential developmental stages. Therefore, to determine the range of phenotypic variation of an organism, it is necessary to consider its genotype, physiological effects of environmental factors, and its developmental plasticity.

Stramonita haemastoma is a common estuarine snail in the northern Gulf of Mexico and along both coasts of Florida. It has received considerable attention with respect to physiological studies on nitrogen metabolism and bioenergetic responses along salinity gradients (Stickle and Howey 1975, Hildreth and Stickle 1980, Garton and Stickle 1980, Findley et al. 1978, Garton 1984, Garton and Stickle 1985, Kapper et al. 1985, Stickle 1985). However, potential genotype specific adaptation to salinity is still undetermined.

Like other osmoconformers, the southern oyster drill adjusts its intracellular osmotic pressure, in part, by altering the concentration of the amino acid pool, primarily alanine and glycine, to adapt to salinity changes (Kapper et al. 1985). These amino acids are derived from multiple metabolic pathways (Bishop et al. 1981, 1983, Deaton et al. 1984, Livingstone et al. 1983, Wickes and Morgan 1976). Genetic variations of the enzymes which are directly or indirectly involved in regulating the amino acid metabolism can be determined by electrophoretic analysis.

Stramonita has recently been revised to a genus separate from Thais (Kool 1987, 1989) on the basis of radular and anatomical differences. The systematic status of S. haemastoma in the Gulf of Mexico has been controversial for many years; it has been identified as two subspecies

(i.e. S. h. canaliculata and S. h. floridana) based on morphological descriptions, radular differences, or geographic locations (Abbott 1974, Andrews 1971, Clench 1947) or as two different forms of S. h. floridana (Walker 1982, Butler 1954, Gunter 1979, Moore 1961).

This study focused on increasing our understanding of salinity adaptation in southern oyster drills. Environmental and genetic effects on salinity adaptation were estimated by salinity-related phenotypic and genotypic variations observed in the studies of salinity tolerance, microcalorimetry, and electrophoresis. In addition, the systematics of Stramonita haemastoma was studied to exclude possible taxonomic differences which may obscure variations in salinity adaptation.

To accomplish these objectives, experiments were divided into three parts: (1) to determine the proper systematic status of Stramonita haemastoma in the southern United States, (2) to determine the salinity-related phenotypic and genotypic variations among populations from high and low salinity habitats, and (3) to determine individual variations in the energetic cost associated with salinity adaptation.

Chapter three of this dissertation was finished first and is in press in Marine Biology with the species name of given as Thais haemastoma. This chapter was accepted for publication prior to the revision of Thais to Stramonita by Kool (1987, 1989). The species name has been changed from T. haemastoma to S. canaliculata after the systematic status of this species was examined by electrophoresis in chapter one. For the sake of consistency, herein, the species name in chapter three is changed to Stramonita canaliculata in this dissertation. Chapter one will be submitted to American Malacological Bulletin and chapter two to Marine Biology.

CHAPTER ONE  
THE SYSTEMATICS OF OYSTER DRILLS, STRAMONITA "THAIS" IN THE SOUTHERN  
UNITED STATES

ABSTRACT

The systematics of Stramonita has previously been based on shell and radular morphology. In this study, electrophoretic results were compared with shell and radular criteria in determining the systematics of Stramonita from 10 localities in the southern United States. S. haemastoma canaliculata, S. h. floridana, and S. rustica can be separated by allozyme data. An UPGMA cluster analysis of Nei's genetic distance (D) shows that S. rustica is differentiated from S. h. canaliculata and S. h. floridana at a distance of 0.46. Based on the Nei's genetic distance between S. h. canaliculata and S. h. floridana (D=0.28) and fixed allelic differences in Fum, LeuAla, Mdh, and 6-Pgdh loci, it is suggested that S. h. canaliculata and S. h. floridana are true species. S. h. canaliculata is predominant in the northern Gulf of Mexico with the exception of South Padre Island (=floridana), while S. h. floridana and S. rustica are predominant on the east coast of Florida. It is hypothesized that S. h. canaliculata is an endemic species, isolated from S. h. floridana by random genetic drift fostered by a few founder individuals originating in the Loop and other currents of the Gulf of Mexico. However, because of the complicated circulation patterns in the Gulf, a low rate of gene-flow among S. h. canaliculata, S. h. floridana, and S. rustica may exist. Although S. h. canaliculata and S. h. haysae are synonyms (Abbott 1974), the older subspecies name canaliculata is suggested for this endemic species (Stramonita canaliculata).

INTRODUCTION

The systematic status of the Thaidid molluscs has been controversial for many years. Recently, Stramonita has been revised to a genus separate from Thais based on the radular and anatomical data (Kool 1987, 1989). Stramonita has at least two representative species

in the southern United States: S. rustica Lamarck and S. haemastoma Linné (Abbott 1974), and Thais has at least one species: T. deltoidea. Stramonita rustica and T. deltoidea have similar distribution patterns ranging from Florida to Brazil. S. haemastoma ranges from Virginia around the Florida peninsula and then westward to Texas (Abbott 1974, Sieling 1960).

Stramonita haemastoma has been listed as Purpura floridana (Conrad 1837) and P. haemastoma floridana Conrad (Johnson 1934) on the basis of shell morphology. Clench (1947) and others (Abbott 1974, Andrews 1971) considered S. haemastoma canaliculata (Gray 1839) and S. haemastoma floridana to be two subspecies of the type S. h. haemastoma Linné, which occurs in the Mediterranean and along the west African coast. In addition, Abbott (1974) suggested that S. h. canaliculata and S. h. haysae are synonyms with the type locality of S. h. canaliculata in China (Yen 1942) and S. h. haysae in Louisiana, United States (Clench 1927). S. h. canaliculata (Gray 1839) reaches a larger total length than S. h. floridana (Conrad 1837), and large adults often bear a double row of strong nodules. The distribution of S. h. canaliculata ranges from western Florida to Texas, while S. h. floridana ranges from Virginia southward around the Florida peninsula then northward to Pensacola, Florida (Abbott 1974, Sieling 1960). S. haemastoma also has been said to occur as a mixed population in the ratio of 6 to 9 canaliculata to 1 floridana in Barataria Bay, LA (St. Amant 1938).

Some investigators have suggested that Stramonita haemastoma canaliculata and S. h. floridana are different forms of the same species, based on shell variation seen within a single population (Walker 1982) or among closely spaced populations in the same bay (Butler 1954, Gunter 1979, Moore 1961), or because reciprocal crosses between these two forms produced normal oviposition and larval development (Butler 1954). Bandel (1984) considered S. h. floridana to be a different form of S. h. haemastoma based on radular and other



developmental characteristics. Cooke (1919), however, treated S. h. floridana as a species (S. floridana Conrad) separate from the original S. h. haemastoma Linné based on radular characteristics.

The study of systematics in molluscs has relied mainly on shell and radular morphology. However, it is suggested that classifications based only on shell or radular characteristics are unreliable (Kool 1987). The development of the techniques of gel electrophoresis during the last decade has provided a valuable tool to systematic studies because it is possible to examine genetic similarities within and between taxa (Avisé 1975). Therefore, electrophoresis was used in this study and compared with shell and radular criteria to determine the proper systematic status of members of Stramonita in the southern United States.

#### MATERIALS AND METHODS

##### Collections

Ten collections of Stramonita were gathered from the northern Gulf of Mexico through the east coast of Florida in their common habitats (Longitude 96°W; Latitude 30°N) (Fig. 1.1). The live snails were: transported to Louisiana State University, kept in recirculating seawater of ambient salinity, and starved for three days to eliminate artifactual electrophoretic patterns due to gut contents. Foot muscle from large snails ( > 2.5cm in total length) or whole soft tissue from small snails ( < 2.5cm) was stored at -70°C for later electrophoresis. Preliminary studies indicated that the mobility of examined allozymes in foot muscle and whole soft tissue were the same. Some shells and buccal masses were saved for shell and radular observations. Voucher specimens were sent to the Museum of Comparative Zoology, Harvard University and the catalog numbers are listed in Table 1.1 and Fig. 1.2.

##### Identification

Species were preliminarily identified using the morphological criteria of Abbott (1974) as Stramonita haemastoma canaliculata, S. h. floridana and S. rustica. S. rustica is small in total length (up to 40

Fig. 1.1 Stramonita sample locations in southern United states. 1. South Padre Island, TX; 2. Red Fish Bay, TX; 3. Caminada Pass, LA; 4. Manila, LA; 5. Henderson Point, MS; 6. Ship Island, MS; 7. Pensacola Bay, FL; 8. Coral Cove State Park, FL; 9. Ponce Inlet, FL; 10. Marineland, FL

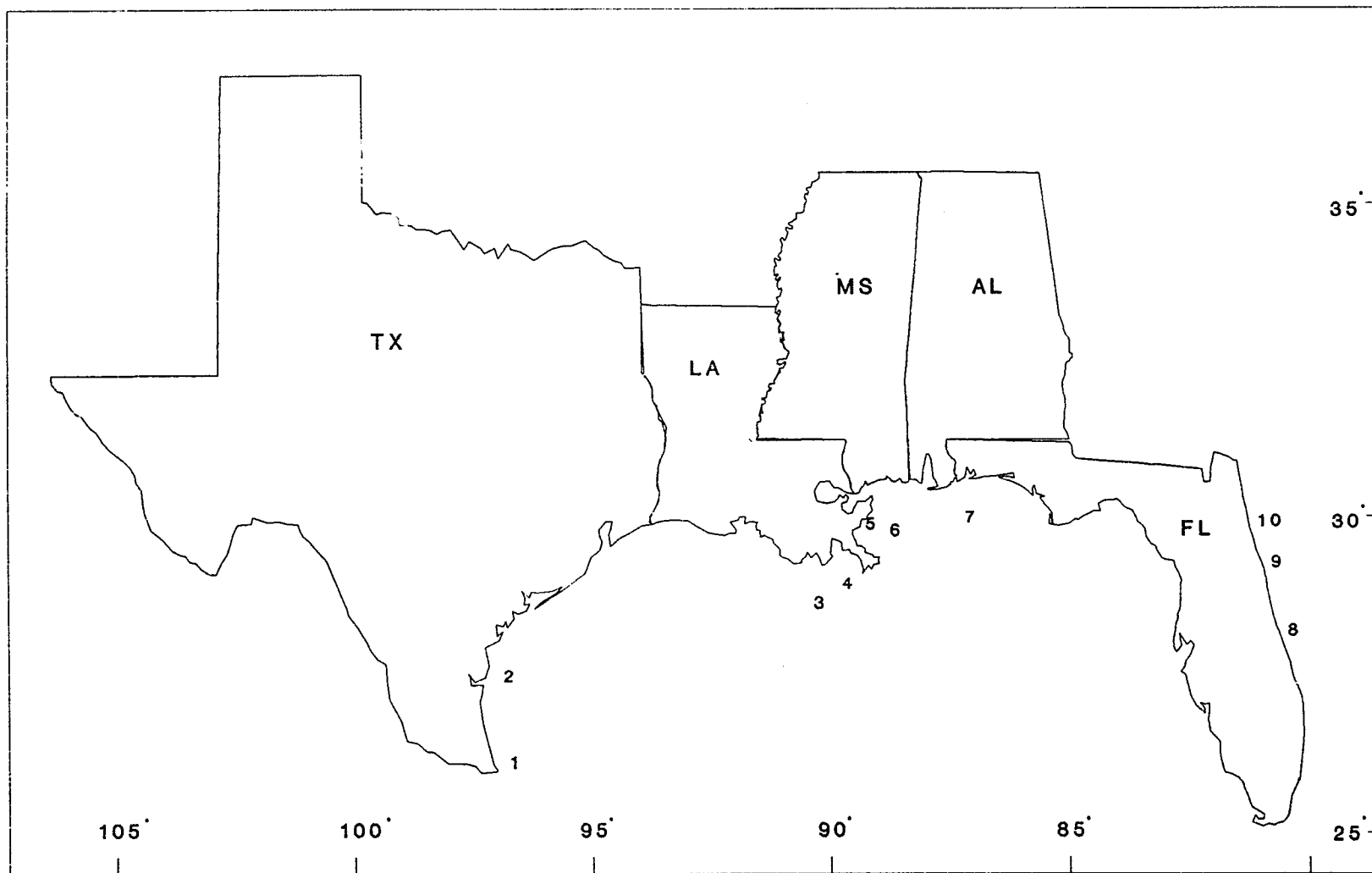
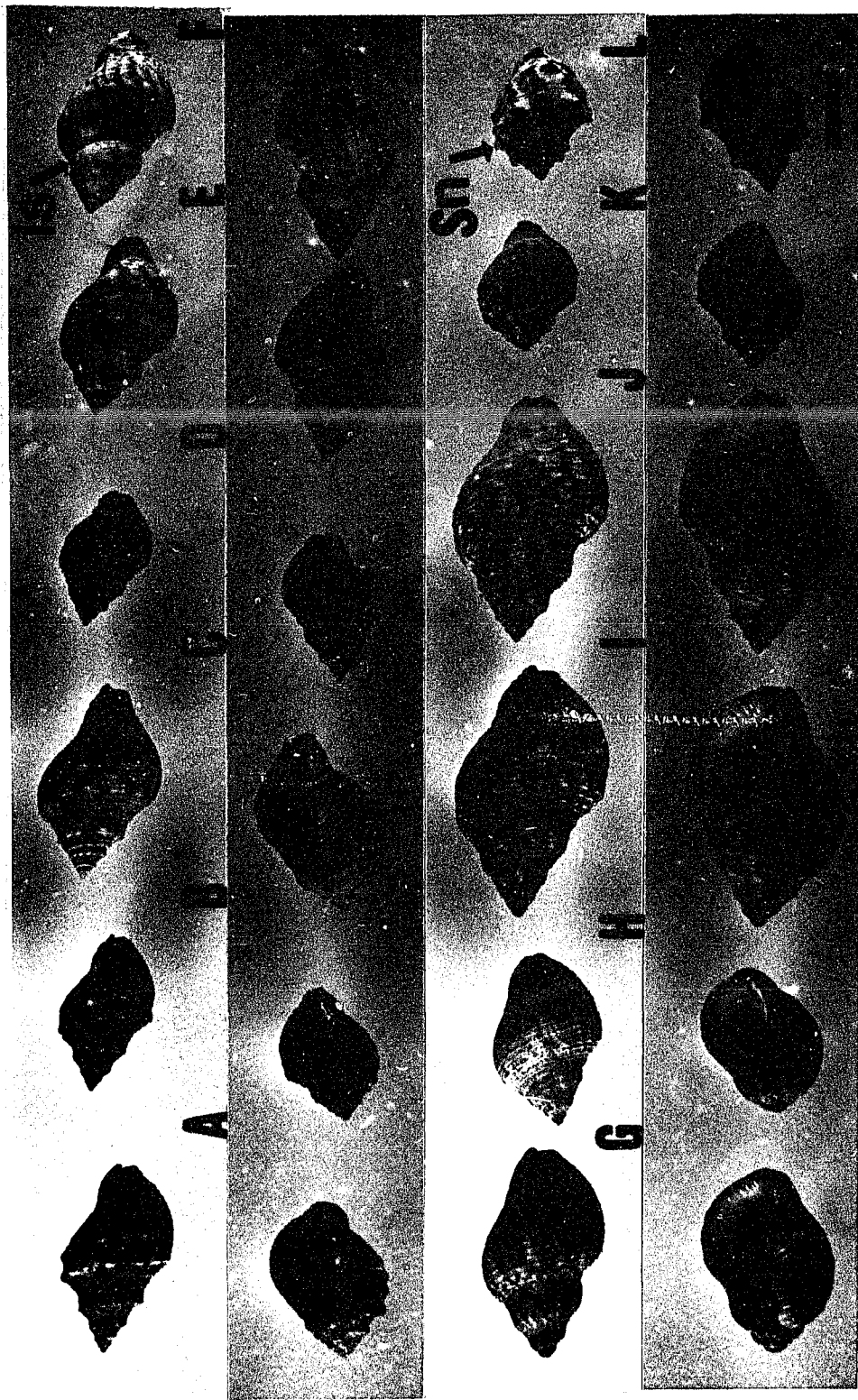


Table 1.1 Collection sites of Stramonita. The species and subspecies are identified as in Abbott (1974) i.e. S. haemastoma canaliculata, S. h. floridana, and S. rustica. Shells are on deposit at the Museum of Comparative Zoology at Harvard University; each collection is identified by the catalog number given below.

Location	Catalog number	Species	Shell length (mm)	Time of collection
South Padre Island, TX	298370	<u>canaliculata</u>	28.2 - 48.8	July, 1989
Red Fish Bay, TX	298371	<u>canaliculata</u>	42.7 - 67.4	July, 1988
Caminada Pass, LA	298372	<u>canaliculata</u>	30.4 - 64.8	October, 1989
Manila, LA	-	<u>canaliculata</u>	26.2 - 62.1	November, 1989
Henderson Point, MS	298373	<u>canaliculata</u>	48.2 - 65.0	October, 1988
Ship Island, MS	298374	<u>canaliculata</u>	27.8 - 59.4	October, 1988
Pensacola Bay, FL	298375	<u>canaliculata</u>	27.8 - 44.7	June, 1988
Marineland, FL	298376	<u>floridana</u>	27.0 - 53.6	August, 1989
Ponce Inlet, FL	298377	<u>floridana</u>	30.4 - 60.1	August, 1989
Coral Cove State Park, FL	298378	<u>rustica</u>	26.4 - 36.3	August, 1989

Fig. 1.2 Representative shells of Stramonita. Catalog number is indicated in parenthesis. (A) S. h. floridana from South Padre Island (298370); (B) possible hybrid of S. h. floridana and S. rustica from South Padre Island (298383); (C) S. h. canaliculata from Caminada Pass (298372); (D) S. h. floridana from Caminada Pass (298379); (E) possible hybrid of S. h. canaliculata and S. h. floridana from Caminada Pass (298380); (F) S. h. canaliculata from Pensacola Bay (298375); (G) S. h. floridana from Marineland (298376); (H) possible hybrid of S. h. canaliculata and S. h. floridana from Marineland (298381); (I) S. h. floridana from Ponce Inlet (298377); (J) possible hybrid of S. h. canaliculata and S. h. floridana from Ponce Inlet (298382); (K) possible hybrid of S. h. floridana and S. rustica from Ponce Inlet (298384); (L) S. rustica from Coral Cove State Park (298378). (scale bar= 2cm; is= indented suture; sn= shoulder nodule)



mm) with two rows of strong shoulder nodules and without an indented suture. S. h. canaliculata is larger in total length (up to 120 mm) with an indented suture and two rows of strong shoulder nodules. S. h. floridana is smaller in total length (up to 50 - 80 mm) with smooth to fine shoulder nodules and without an indented suture. Because individuals grow larger as they age, body size is not useful as a criterion to distinguish subspecific differences between S. h. canaliculata and S. h. floridana. Suture indentation and the presence of two rows of shoulder nodules were therefore used to distinguish the two subspecies.

Only a few specimens had both an indented suture and shoulder nodules, characteristics of Stramonita haemastoma canaliculata. Most of the specimens had either an indented suture or shoulder nodules. By convention, specimens with either an indented suture or shoulder nodules, were designated S. h. canaliculata. Also, not all of the individuals from a locality always met either canaliculata or floridana criteria. Therefore, if more than 50% of the individuals of a collection fit the criteria of the subspecies, that name has been adopted for the collection.

#### Shell morphology

Six shell measurements were scored for snails from six collections (South Padre Island, TX (n= 72), Caminada Pass, LA (n= 24), Pensacola Bay, FL (n= 10), Coral Cove State Park, FL (n= 5), Ponce Inlet, FL (n= 21), and Marineland, FL (n= 35)). Shell length, width, and height (the vertical maximum distance when the snail is in a normal crawling position) were measured by vernier calipers ( $\pm 0.1\text{mm}$ ). To eliminate discrepancies resulting from size differences among collections, ratios of shell length, width and height were used in the data analysis. The degree of suture indentation and shoulder nodule prominence were scored qualitatively. Sutures were rated from 0 (no indentation, Fig. 1.2 F) to 3 (strongly indented, Fig. 1.2 I). Shoulder nodules were rated from

0 (smooth nodules, Fig. 1.2 I) to 5 (prominent nodules, Fig. 1.2 L).

### Radular morphology

Snail radulae from four collections (Caminada Pass, LA (n= 11), Ponce Inlet, FL (n= 4), Coral Cove State Park, FL (n= 4), and Marineland, FL (n= 13)) were examined. Each buccal mass was soaked overnight in a 10% KOH solution. Each radula was rinsed in distilled water and ultrasonically cleaned for 30 seconds. It was dried, mounted and sputter-coated with gold-palladium. The specimens were then examined with a Hitachi S-500 scanning electron microscope at 25 KV. Thirteen radular characteristics were studied, twelve of which follow Kool (1987), i.e. the central cusp morphology (also follows Radwin and Wells (1968)), the longitudinal cavity in central cusp, the denticle between central and lateral cusp, the lateral cusp orientation, the outer denticle(s) on lateral cusp, the area between lateral cusp and side of rachidian, the marginal denticles, the position of lateral cusp relative to marginal edge, the marginal cusp, the lateral extension of rachidian base, the lateral tooth, and the lateral tooth length. The number of marginal denticles was also counted. In all the radular samples, ten characteristics were similar to Stramonita "Thais" haemastoma as described by Kool (1987). Accordingly, only the central cusp morphology, the comparison of the lateral tooth length to rachidian width, and the number of marginal denticles were used in this study (Fig. 1.3).

### Allozyme methods

Foot muscle or whole soft tissues were homogenized in a Tekmar tissumizer with an equal volume of 10 mM Tris-HCl buffer (pH 7.0) containing 1% Triton X-100. Homogenates were centrifuged at 17,000 g for 15 min and the supernatants were stored at -70°C. Gels contained 11 or 12.5% starch (Table 1.2). Schaal and Anderson (1974) and Harris and Hopkinson (1978) have described general methods of horizontal starch gel electrophoresis and enzyme staining which, with the following



modifications and additions, were used (Table 1.2). The TC3 7.0 buffer was modified from Ahmad et. al. (1977) with 1 mM  $\text{MgCl}_2$  added to both the gel and electrode buffers. The TC4 7.0 buffer was modified from Ahmad et al. (1977) with 0.005% NADP (w:v) added to the gel and electrode buffers. The alanopine dehydrogenase (AlaDH) staining method was modified from Dando et al. (1981) in that 70 mg L-alanine, 2 mg NADH, and 6 mg pyruvate were added to 25 ml 0.1 M Tris-HCl buffer at pH 7.5. The LAP1 & 2 staining method was modified from Murdock et. al. (1975) in that the sliced gel was pre-incubated in the substrate solution (25 mg L-Leucine 4-methoxy- $\beta$ -naphthylamide for LAP1, or 25 mg DL-alanine  $\beta$ -naphthylamide for LAP2 in 50 ml 0.2 M Tris-malate buffer, pH 6.4) for 45 min, then stained with 50 mg Fast black K in 50 ml 0.2 M Tris-malate buffer at pH 6.4. All the fluorescent stains in that substrate solutions were discarded after 30 sec incubation of gel slices and the bands were then directly observed under UV light. Details of the electrophoretic recipes for all buffers and stains are in Appendix 1.1.

Multiple loci encoding the same enzyme (isozymes) were designated by consecutive numbers, with "1" denoting the slowest migrating isozyme. Of the twenty-one loci identified, 16 could be consistently scored in this study. Alleles were scored by designating the most common allele as 100. All other alleles were numbered according to their relative anodal distance from the most common allele. All allozyme systems exhibited anodal migration except Est1 and Gpt1.

#### Statistical analyses

Principal component analysis was used separately in the shell, radular, or electrophoretic data set to determine whether the variables could be used to separate collections of Stramonita into groups and to identify which variables contribute to the separation. Principal component analysis could not separate Stramonita species into groups by shell and radular data. Therefore, only the electrophoretic principal component analysis is presented in this study. A separation among

Table 1.2 Enzymes assayed by electrophoresis in Stramonita.  
(E.C. #: enzyme commission number; <sup>a</sup>: total number of  
presumed loci detectable; <sup>b</sup>: unscored locus in present  
study; <sup>c</sup>: the percentage of starch used)

Enzyme (E.C. #)	Genetic symbol	Number of loci <sup>a</sup>	Starch (%) <sup>c</sup>	Buffer system
Adenylate kinase (2.7.4.3)	<u>Adk</u>	2, (1) <sup>b</sup>	11%	TC1 8.0
Alanopine dehydrogenase (1.5.1.17)	<u>Aladh</u>	1	11%	TC4 6.7
Esterase (3.1.1.1)	<u>Est</u>	2	12.5%	PC 7.0
Fumerase (4.2.1.2)	<u>Fum</u>	1	12.5%	PC 7.0
Glutamate dehydrogenase (1.4.1.2)	<u>Gdh</u>	1	12.5%	TM 7.4
Glutamate-oxaloacetate transaminase (2.6.1.1)	<u>Got</u>	2, (1)	11%	TC1 8.0
Glutamate-pyruvate transaminase (2.6.1.2)	<u>Gpt</u>	2	12.5%	TM 7.4
Leucine aminopeptidase (3.4.11 or 13)	<u>Lap</u>	2, (1)	11%	TC2 7.0
di-Peptidase (specific to leu-ala) (3.4.11 or 13)	<u>LeuAla</u>	1	11%	TC2 7.0
Malate dehydrogenase (1.1.1.37)	<u>Mdh</u>	2, (1)	11%	TC4 6.7
Phosphoglucomutase (2.7.5.1)	<u>Pgm</u>	2, (1)	11%	TC4 6.7
Pyruvate kinase (2.7.1.40)	<u>Pk</u>	1	12.5%	TM 7.4
6-Phosphogluconate dehydrogenase (1.1.1.44)	6- <u>Pgdh</u>	1	11%	TC3 7.0
Xanthine dehydrogenase (1.1.1.204)	<u>Xdh</u>	1	12.5%	TM 7.4

groups is drawn by comparing the variances of all the variables within- and among-collections. The first through sixth highest principal components were reported since they have the heaviest contribution to the separation of within- and among-collections. For the electrophoretic data, each allele was treated as a separate variable, with the number of copies of the allele (0, 1, or 2) in that individual as the value of the variable. Nei's genetic distance coefficients (D) (1978) were calculated and clustered by the unweighed pair-group method with arithmetic means (UPGMA) algorithm (Sneath and Sokal 1973). These analyses were performed with SAS (SAS Institute, Inc. 1982).

## RESULTS

Snails with different degrees of suture indentation and shoulder nodule prominence are found within some collections, e.g. Caminada Pass (Fig. 1.2). However, shell shape is quite uniform in the South Padre Island, Pensacola Bay, Marineland, and Ponce Inlet collections. The ranges of the quantitative and qualitative measurements in shell morphology overlapped considerably among collections (Table 1.3). For example, snails from South Padre Island (i.e. Stramonita haemastoma floridana) and Coral Cove State Park (i.e. S. rustica) have more prominent first shoulder nodules (=2 to 3) than other collections (i.e. S. h. canaliculata and S. h. floridana) (=0 to 1). Principal component analysis suggests no separation exists among collections (results not shown) although the first (35%), second (26%), and third (17%) principal components account for 78% of the total variation among individuals. The first and second shoulder nodules are the primary variables in the first principal component and the ratio of length to width and height are the primary variables in the second principal component.

Thirty-two snails from four collections were selected for study of radular characteristics by photomicrograph. The radulae of Stramonita haemastoma canaliculata are similar to that of S. h. floridana (Fig. 1.3 A, B). However, the lateral cusp serration in Ponce Inlet collection is

Table 1.3 Shell measurements of six collections of Stramonita. Species and percent major species are determined by electrophoretic study. (quantitative measurements: mean $\pm$ S.E.; qualitative measurements: median/ranges)

Site	South Padre Island	Caminada Pass	Pensacola Bay	Coral Cove State Park	Ponce Inlet	Marineland
Sample size (n)	72	24	10	5	21	35
Species	<u>floridana</u>	<u>canaliculata</u>	<u>canaliculata</u>	<u>rustica</u>	<u>floridana</u>	<u>floridana</u>
Major species(%)	98	83	100	100	90	91
Quantitative Measurement						
Length (mm)	38.32 $\pm$ 0.50	40.49 $\pm$ 1.41	37.39 $\pm$ 1.43	32.14 $\pm$ 1.84	48.82 $\pm$ 1.85	43.25 $\pm$ 0.81
Length/length	0.52 $\pm$ 0	0.50 $\pm$ 0	0.53 $\pm$ 0.01	0.58 $\pm$ 0.01	0.54 $\pm$ 0	0.53 $\pm$ 0.01
Height/length	0.57 $\pm$ 0	0.59 $\pm$ 0.01	0.57 $\pm$ 0.01	0.64 $\pm$ 0.01	0.60 $\pm$ 0.01	0.61 $\pm$ 0.01
Qualitative Measurement						
Suture (0-3)	0/0	1/0-2	3/1-3	0/0	0/0	0/0-1
1st nodules(0-5)	2/0-4	1/0-2	1/0-3	3/2-5	0/0-4	0/0-1
2nd nodules(0-5)	1/0-3	0/0	0/0-1	2/1-3	0/0-2	0/0

Fig. 1.3 Scanning electron micrographs of the radula of Stramonita. (A) S. haemastoma canaliculata from Caminada Pass; (B) S. h. floridana from Marineland; (C) S. h. floridana from Ponce Inlet; (D) S. rustica from Coral Cove State Park; (E) possible hybrid of S. h. canaliculata and S. h. floridana from Marineland; (F) possible hybrid of S. h. floridana and S. rustica from Ponce Inlet. (scale bar= 50 $\mu$ m; cc= central cusp; lc= lateral cusp; lt= lateral tooth; md= marginal denticle; r= rachidian)

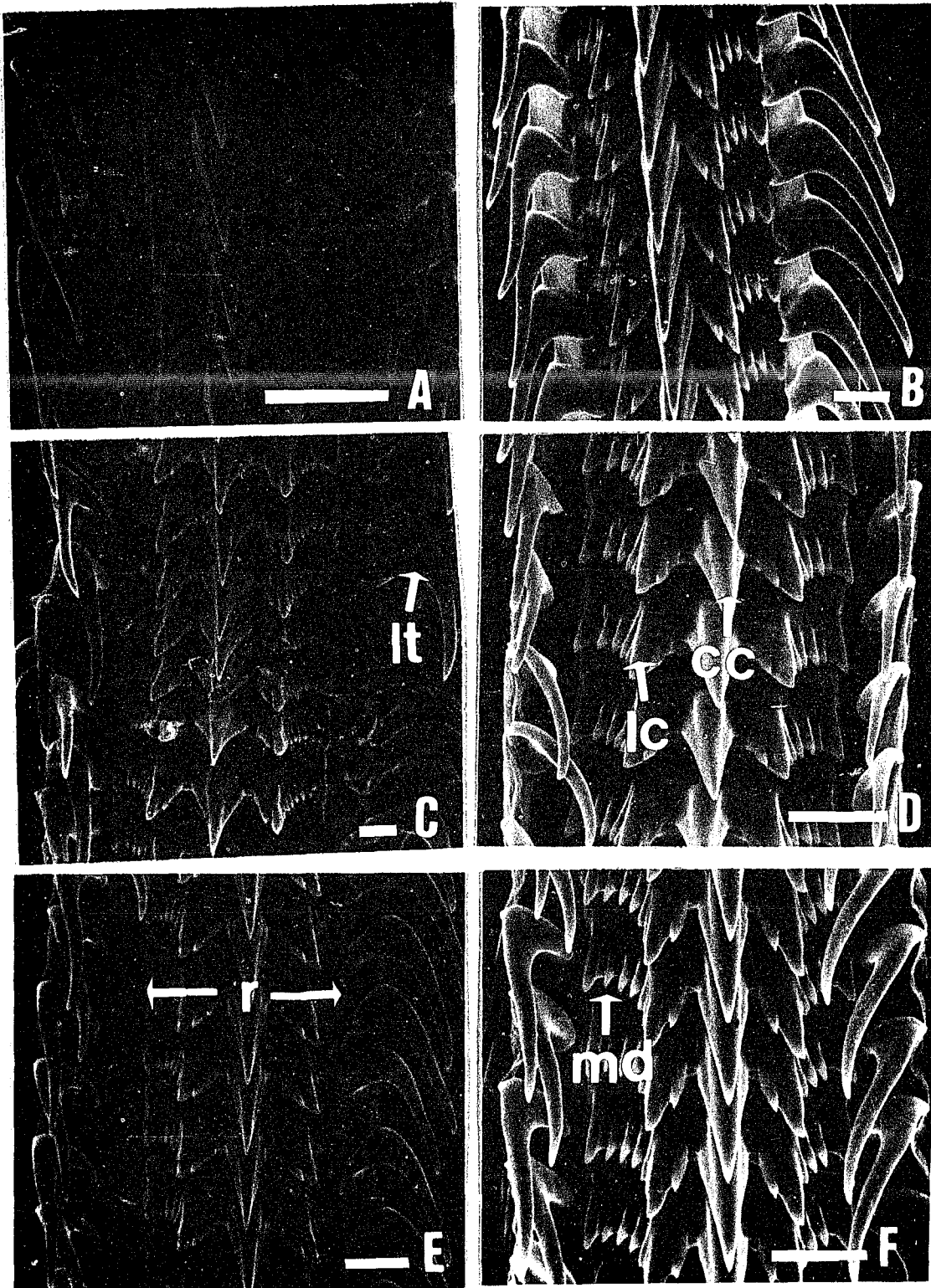


Table 1.4 Radular variations of four collections of Stramonita.

Site Marineland	Caminada Pass	Coral Cove State Park	Ponce Inlet	
Species	<u>canaliculata</u>	<u>rustica</u>	<u>floridana</u>	<u>floridana</u>
Sample size (n)	11	4	4	13
The comparison of lateral tooth length to rachidian width <sup>a</sup>	a	b	a	a
Central cusp morphology <sup>b</sup>	a,b	a	b	b
The number of denticles (mean/range)	2.7/1-4	2.3/1-3	3.5/3-4	2.5/2-3

- <sup>a</sup>: lateral tooth length which is longer or equal to rachidian width (=a) (Fig. 1.3B) or shorter than rachidian width (=b) (Fig. 1.3D).  
<sup>b</sup>: central cusp tapers equally to its base (=a) (Fig. 1.3D) or has a tapering crown with the sides being parallel toward its base (=b) (Fig. 1.3A).

different from Marineland although both are S. h. floridana (Fig. 1.3 B, C). Coral Cove State Park collection is S. rustica (Fig. 1.3 D) by comparing the radular morphology of snails with radulae from other studies (Cooke 1918, Bandel 1984). S. rustica and S. haemastoma distinctly differ in that the lateral tooth length is shorter than the rachidian tooth width in S. rustica but not in S. haemastoma (Table 1.4 and Fig. 1.3 B, D). Principal component analysis suggests that S. rustica can be separated from S. haemastoma; however, no apparent separation exists between S. h. canaliculata and S. h. floridana (results not shown). The first (63%) and second (30%) principal components account for 93% of the total variation among individuals. The comparison of lateral tooth length to rachidian width and central cusp morphology are the primary variables in the first principal component and the number of denticles is the primary variable in the second principal component.

Three of the 16 enzyme loci investigated are monomorphic (using the 0.95 criterion of polymorphism): Est1, Gpt1, and Xdh. Mean allelic frequencies at each locus for each collection are in Appendix 1.2. Based on the similarity in the predominant allelic frequencies in those polymorphic enzyme loci, ten collections are separated into three groups. The first group is Stramonita rustica from Coral Cove State Park, the second group is S. haemastoma floridana which was taken from South Padre Island, Ponce Inlet, and Marineland, and the third group is S. h. canaliculata from the remaining collections. The predominant alleles of Fum, Lap1, Mdh, and LeuAla loci are the same in S. h. floridana and S. rustica, while, S. h. canaliculata is different (Appendix 1.2). However, S. rustica is different from S. h. floridana and S. h. canaliculata in the predominant alleles of Aladh, Gdh, Got2, and Gpt2 loci. In addition, the predominant alleles are different from each other in the 6-Pgdh locus. The predominant alleles in S. rustica, S. h. floridana, and S. h. canaliculata are 6-Pgdh<sup>88</sup> (=0.600), 6-Pgdh<sup>81</sup>

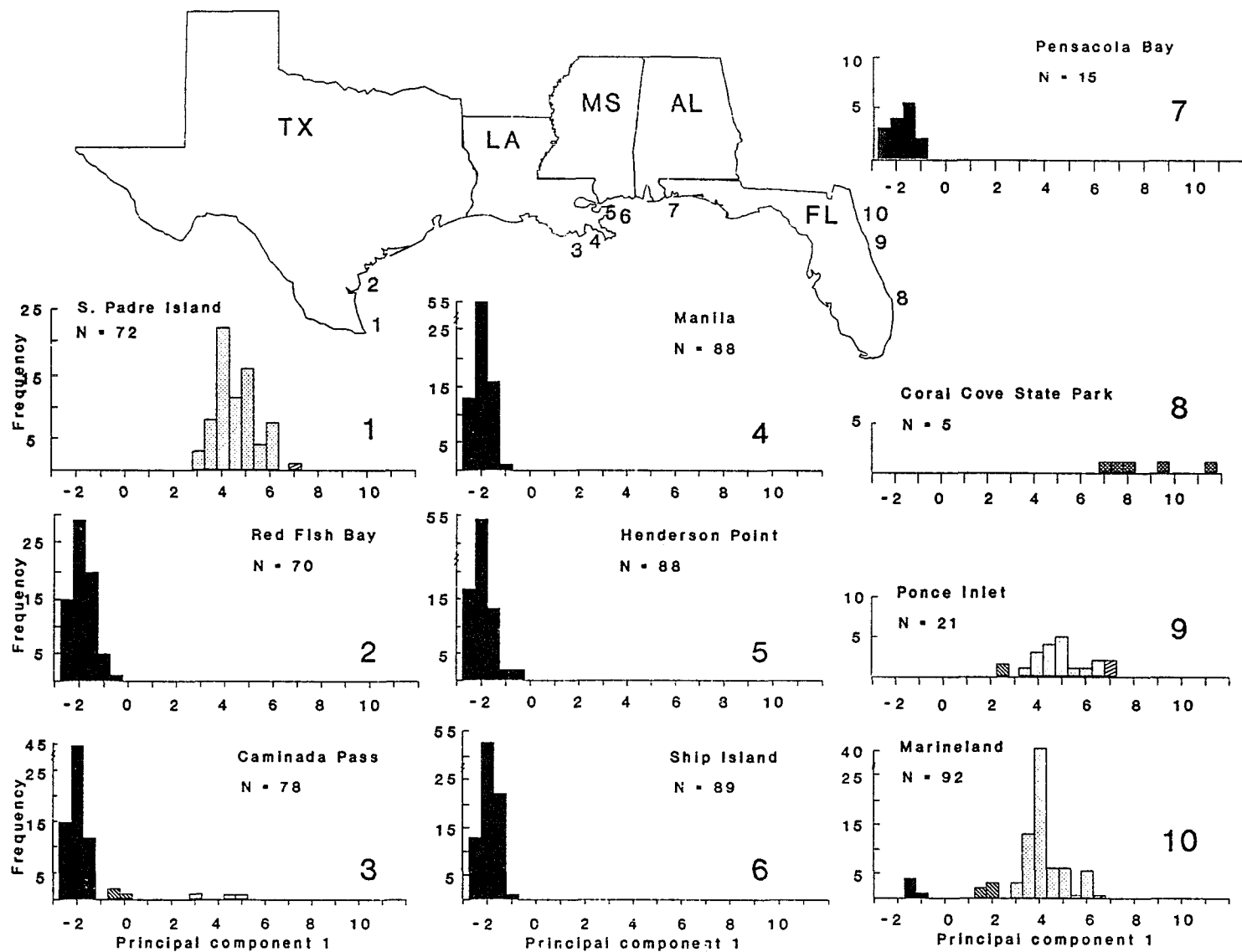


(=0.607), and 6-Pgdh<sup>100</sup> (=0.979), respectively. Although enzyme loci in Lap2, Mdh2, and Pgm1 are also different among S. rustica, S. h. floridana, and S. h. canaliculata, the results are difficult to interpret and are not included in the data analysis.

Fixed allelic differences between Stramonita haemastoma canaliculata and S. h. floridana were observed at four (i.e. Fum, LeuAla, Mdh, and 6-Pgdh) of the sixteen enzyme loci (Appendix 1.2). The predominant allele of Fum is Fum<sup>100</sup> in S. h. canaliculata (mean allelic frequency = 0.996) and Fum<sup>85</sup> in S. h. floridana (= 0.961). LeuAla<sup>100</sup> is predominant in S. h. canaliculata (= 0.992), while, LeuAla<sup>88</sup> (= 0.876) and LeuAla<sup>75</sup> (= 0.085) are predominant in S. h. floridana. Mdh and 6-Pgdh also exhibit similar fixed allelic differences between S. h. canaliculata and S. h. floridana.

Principal component analysis separated the electrophoretic data into three groups. The major grouping (variation among collections) is due to the first principal component. Therefore, only first principal component data are presented and plotted separately by collections (Fig. 1.4). The three groups are: (1) S. rustica from Coral Cove State Park, FL which constitutes the right peak; (2) S. haemastoma floridana from the collections at South Padre Island, TX, Ponce Inlet, and Marineland, FL which constitutes the middle peak; and (3) S. h. canaliculata from the remaining collections which constitutes the left peak. This grouping is generally in accordance with previous suggestions based on shell morphology and the distribution of Stramonita spp. by Abbott (1974). One discrepancy is the collection from South Padre Island which should correspond to S. h. canaliculata based on shell morphology instead it displays S. h. floridana allelic frequencies (Fig. 1.4). In addition, the first to the sixth principal components (15, 10, 5, 4, 4, and 3%, respectively) account for 41% of the total variation among individuals and collections. Alleles of the enzyme loci of Adk, Fum, LeuAla, Mdh, and 6-Pgdh are primarily variables which determine the

Fig. 1.4 Stramonita sample locations in southern United States and the frequency of the first principal component values for each collection. (N= sample size; ■ = S. haemastoma canaliculata; □ = S. h. floridana; ▨ = S. rustica; ▩ = possible hybrids of S. h. canaliculata and S. h. floridana; ▧ = possible hybrids of S. h. floridana and S. rustica)



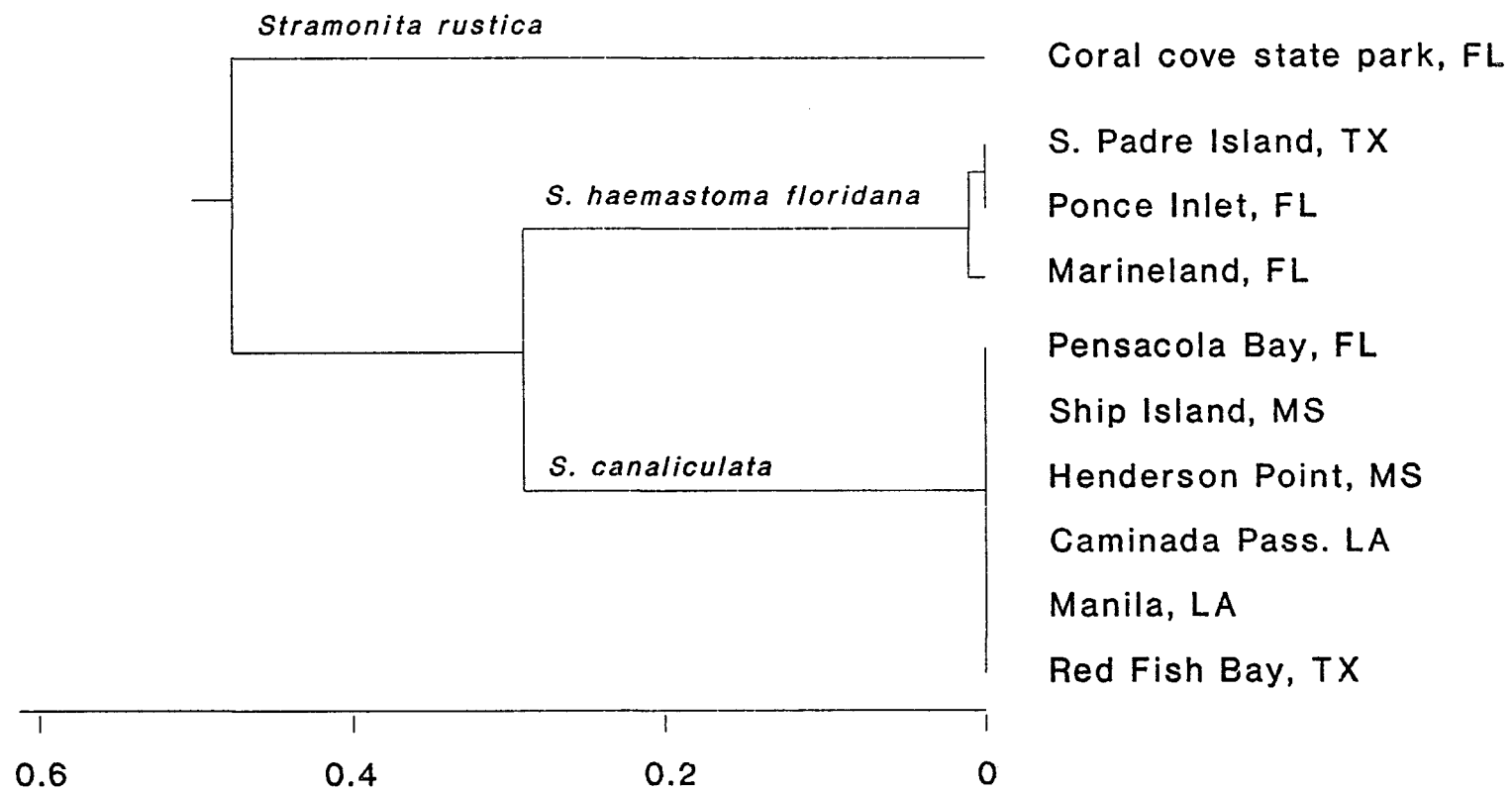
first principal component and Adk, Adh, Gdh, Got2, Gpt2, and 6-Pgdh are primarily variables for the second principal component.

An UPGMA cluster analysis was determined by Nei's (D) genetic distance values. Stramonita rustica is separated from S. haemastoma floridana and S. h. canaliculata at a distance of 0.46 (Fig. 1.5). S. h. floridana and S. h. canaliculata are differentiated at a distance of 0.28. Only minor differentiation exists among the three S. h. floridana populations ( $D < 0.002$ ); and the six S. h. canaliculata populations exhibit little inter-population variation ( $D = 0$ ). Comparison with other marine invertebrate genetic distance values and fixed allelic differences indicate that the difference between S. h. floridana and S. h. canaliculata could be interpreted on specific rather than subspecific level.

Based on the first principal component values for allozyme data, South Padre Island, Caminada Pass, Ponce Inlet, and Marineland collections appear to be a mixture of Stramonita haemastoma canaliculata and S. h. floridana or of S. h. floridana and S. rustica (Fig. 1.4). For example, at Caminada Pass, the majority are S. h. canaliculata with few S. h. floridana (<4% which is determined by the numbers of S. h. floridana/total sample size) and possible hybrids of the two subspecies (<4%). Additionally, considering the small sample size ( $n=5$ ) and the wide range of the first principal component in Coral Cove State Park collection (S. rustica), it is possible that more than one species is present.

Several possible hybrids were selected based on allozymic pattern from South Padre Island, Caminada Pass, Ponce Inlet, and Marineland collections to identify any salient features in shell or radular morphology. For example, in Caminada Pass, the shell morphology of Stramonita haemastoma floridana and the possible hybrids of S. h. canaliculata and S. h. floridana are similar to S. h. canaliculata (Fig. 1.2 D, E). Similarly, no differences are observed in possible hybrids

Fig. 1.5 The UPGMA cluster analysis of Nei's genetic distances (D) among collections of Stramonita.



from South Padre Island (Fig. 1.2 B), Marineland (Fig. 1.2 H), and Ponce Inlet (Fig. 1.2 J, K). S. h. canaliculata, S. h. floridana, and their possible hybrids are similar in radular morphology (Fig. 1.3 A, B, E). However, the radula of a possible hybrid of S. h. floridana and S. rustica from Ponce Inlet is similar to S. h. floridana (Fig. 1.3 B, F).

Based on the electrophoretic data, shell measurements were reanalyzed by grouping the collections (using only the major species of the collection) into Stramonita haemastoma canaliculata, S. h. floridana, and S. rustica (Table 1.5). However, the ranges of these qualitative characteristics (e.g. 1st shoulder nodules) overlap greatly among species. For example, the first shoulder nodules is from 0 to 3 in S. h. floridana, from 0 to 4 in S. h. canaliculata, and from 2 to 5 in S. rustica (Table 1.5). Therefore, the degree of suture indentation and shoulder nodule prominence are not a good criteria to determine species.

#### DISCUSSION

Allozyme studies, comparative anatomy and shell morphology are often used to assess relationships among molluscan individuals, species, genera and higher taxa. Davis (1983) studied the taxonomy in the family of Unionidae (bivalves) by these methods and found the ability to differentiate taxa was: allozyme data > comparative anatomy > shell morphology. In this study, allozyme data are also more powerful than radular or shell characteristics in separating species and subspecies of Stramonita rustica, S. haemastoma canaliculata, and S. h. floridana. The difference between S. h. canaliculata and S. h. floridana is on the species level based on Nei's genetic distance ( $D = 0.28$ ) and fixed allelic differences in Fum, LeuAla, Mdh, and 6-Pgdh loci.

Many studies have suggested that shell morphology in gastropods is related to environmental factors, e.g. shell size (Etter 1989) and lamellation (=sculpture which consists of parallel spiral ridges on the shell) (Largen 1971). In Nucella lapillus, lamellation decreases with

Table 1.5 Shell measurements of three Stramonita species based on electrophoretic separation. (quantitative measurements: mean $\pm$ S.E.; qualitative measurements: median/ranges)

Species	<u>canalicula</u>	<u>floridana</u>	<u>rustica</u>
Sample size (n)	30	122	5
Quantitative measurement			
Length (mm)	39.61 $\pm$ 1.12	41.45 $\pm$ 0.59	32.14 $\pm$ 1.84
Width/length	0.58 $\pm$ 0.05	0.58 $\pm$ 0	0.64 $\pm$ 0.01
Height/length	0.51 $\pm$ 0.05	0.52 $\pm$ 0	0.57 $\pm$ 0.01
Qualitative measurement			
Suture (0-3)	1/0-3	0/0-1	0/0
1st nodules(0-5)	1/0-3	1/0-4	3/2-5
2nd nodules(0-5)	0/0-1	1/0-3	2/1-3



an increase in the degree of exposure to wave action (Largen 1971). Also, the shell shape variation of N. lapillus and N. emarginata from short and broad to tall and narrow is related to the degree of exposure to wave action (Crothers 1983, Kitching 1976); however, in some areas, N. lapillus are usually elongated regardless of the exposure of their habitats (Crothers 1983). Therefore, it has been postulated that the shell shape of N. lapillus is a result of the mixture of several genetically distinct populations into a single polymorphic population, and within that population some traits have responded to the selective effects of wave actions while others have not (Crothers 1983). In addition, Palmer (1984, 1985) indicated that the phenotype of lamellation in N. emarginata is controlled by a sculpture dominant two-allelic locus and modified by the growth environment of juveniles (Palmer 1984, 1985). Therefore, intraspecific variability in shell morphology is determined by a genetic program that is modified by environmental factors. Our results also indicate that it is difficult to identify Stramonita species based on shell morphology alone.

Stramonita rustica is primarily separated from S. haemastoma by the radular characteristics of different central cusp shapes and a shorter lateral tooth (Table 1.4 and Fig. 1.3 A - D). However, there is no detectable difference between S. h. canaliculata and S. h. floridana in radular characteristics (Fig. 1.3 A, B). It has been suggested that the anatomies of marine prosobranch radulae are related to phylogeny, food source, ontogeny and other factors (Hickman 1980, Kool 1987). For example, although radular shape is not changed by the regeneration processes in S. h. canaliculata, the regenerated structures will be slightly smaller in size than their original complements (Roller et al. 1984). Radular structures are abraded by wear as a result of feeding on bivalve prey in S. h. canaliculata or feeding on tougher algae in Littorina littorea (Bertness et al. 1983). Also, due to a change of prey choice, juvenile S. h. floridana radula may change to a S.

haemastoma form (Bandel 1984). Therefore, it is important to distinguish radular differences which are due to phylogeny or environmental factors when using these criteria to determine species differences.

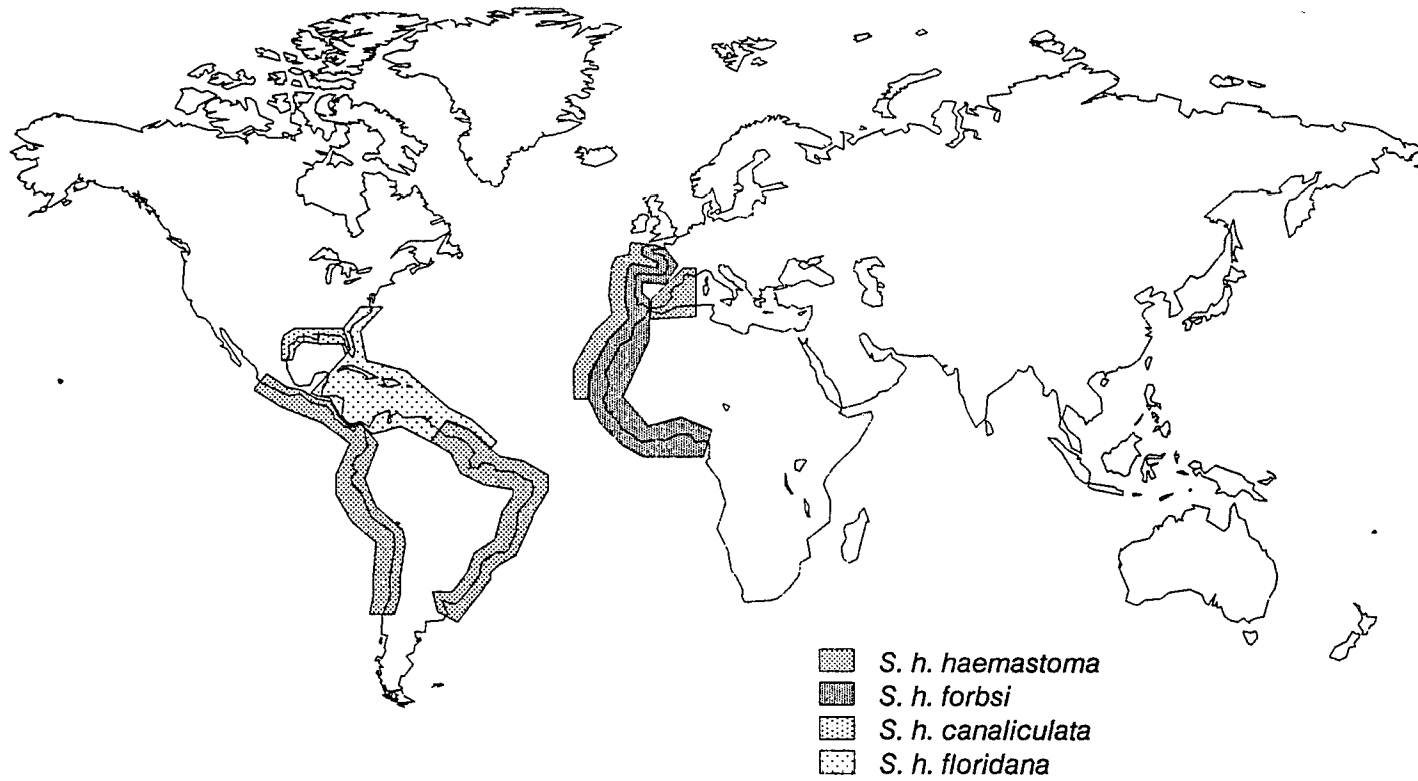
Based on electrophoretic data, clear differences in allelic frequencies occur between Stramonita rustica and S. haemastoma canaliculata in 9 of the 16 loci (=56%) and between S. h. canaliculata and S. h. floridana in 4 of the 16 loci (25%). Similar results have been reported in freshwater snails where the genetic differences between Oncomelania hupensis hupensis and O. h. quadrasi were measurably present in 7 of 21 loci (33%) with Nei's genetic distance at 0.62 (Woodruff et al. 1988). Based on the genetic data, Woodruff et al. suggested that O. h. hupensis and O. h. quadrasi are different species.

Thorpe (1983) found that over 95% of the studies using Nei's genetic distance in invertebrates exhibited congeneric species ranging from 0.19 to 2.59. For example in oysters, the genetic distance between congeneric Crassostrea ranges from 0.32 to 2.29 and congeneric Saccostrea from 0.17 to 0.53 (Buroker et al. 1979). In freshwater clams of Unionidae, the confamily genetic distances range from 0.21 to 1.51 (Davis 1983). It is also suggested that the fixed allelic differences at more than 20% of assayed enzyme loci can be considered separate species (Richardson et al. 1986). Here, based on the genetic distance between S. h. canaliculata and S. h. floridana ( $D = 0.28$ ) and the fixed allelic differences (25%), we suggest that S. h. canaliculata and S. h. floridana should be separated at the species level.

Four subspecies are involved in the Stramonita haemastoma complex: S. h. haemastoma, S. h. forbesi, S. h. canaliculata (now S. canaliculata), and S. h. floridana (Clench 1947, Fig. 1.6). The subspecies S. h. haemastoma is found in the east Atlantic (from France through western Mediterranean and along the African coast to Senegal), west Atlantic (from Trinidad south to Uruguay), and east Pacific Oceans

Fig. 1.6 World distribution of four subspecies of Stramonita  
haemastoma.

# WORLD DISTRIBUTION OF *Stramonita haemastoma*



(from Mexico south to central Chile). S. h. forbesi is an eastern Atlantic subspecies ranging from France to the Congo. S. h. floridana and S. h. canaliculata are western Atlantic subspecies. S. h. floridana ranges from Virginia along the Central American coast to Brazil. S. h. canaliculata ranges from western Florida to Texas.

From plankton samples, Scheltema (1971) estimated that the pelagic larval stage in Stramonita haemastoma is 90 days therefore trans-oceanic dispersal by the North Atlantic Drift and the North and South Equatorial Currents is a common event (Scheltema 1972). Due to the low abundance of larvae, Scheltema (1972, 1975) suggested that the rate of gene-flow is not high and the subspecies taxa are valid in the S. haemastoma complex.

In this study, the distribution of Stramonita in the southern United States is similar to that reported by Clench (1947), Abbott (1974), and Scheltema (1971, 1972); S. canaliculata is predominant in the Gulf of Mexico while S. haemastoma floridana and S. rustica are predominant along the east coast of Florida. However, based on the allelic frequencies, the South Padre Island collection primarily consists of S. h. floridana instead of S. canaliculata. In addition, some possible hybrids of S. canaliculata and S. h. floridana and of S. h. floridana and S. rustica are found at South Padre Island, Caminada Pass, Ponce Inlet, and Marineland collections.

The distribution of northern American fiddler crabs (Uca spp.) (Barnwell and Thurman 1984) and stone crabs (Menippe spp.) (Williams and Felder 1986) within the Gulf of Mexico appears to correlate with the surface currents and sedimentary patterns. Current circulation in the Gulf of Mexico is complex, but the largest influence is the Loop Current and its associated eddies (Holt et al. 1982, Leipper 1954). The Loop Current is formed as the Yucatan current enters the central Gulf through the Yucatan Straits. Subsequently, it exits the Gulf by combining with other water masses to form the Gulf Stream and then flows north along

the east coast of Florida. The results of drift bottle experiments indicated that bottles were returned from the Texas coast to the east coast of Florida with the highest return rate occurring around Key West up to the east coast of Florida (Holt et al. 1982). Therefore, it is possible for the larvae of Stramonita to be carried into or flushed out of the Gulf of Mexico by these circulation patterns.

Stramonita rustica has a long-term planktotrophic larval stage (D'Asaro 1970, Spight 1977) and the species ranges from Bermuda through Florida to the West Indies (Morris 1973). This range to a large extent overlaps the distribution of S. haemastoma haemastoma and S. h. floridana along the Florida coast and in the Caribbean Sea. In addition, S. h. canaliculata may be introduced to Florida's coast by the circulation of the Loop Current and Gulf Stream. Therefore, it is possible for gene-flow to exist among these species and subspecies. Consequently, hybridization among them may occur, such as in South Padre Island, Caminada Pass, Ponce Inlet and Marineland collections. However, due to the small sample size and wide range in the first principal component in S. rustica (Coral Cove State Park collection), it is hard to determine whether this collection is pure S. rustica or a mixture of S. rustica with others.

A population which passes through a period of reduced numbers (e.g. founder effect) will lose some of its alleles with a consequent decrease in genetic variability (Ferguson 1980). In this study, Stramonita haemastoma canaliculata appears to exhibit less allelic frequency variation than S. h. floridana (Appendix 1.2). It is hypothesized that S. h. canaliculata is an endemic species which may have evolved from S. h. floridana through random genetic drift fostered by a few founder individuals originating from the circulation currents in the Gulf of Mexico. However, a minute gene-flow with S. h. floridana or other Stramonita spp. may be provided by currents through the Yucatan Straits to the Louisiana coast. Therefore, because of circulation

patterns, S. h. canaliculata at Caminada Pass has a greater chance to interact with S. h. floridana and become a mixed collection. The introduction of S. h. floridana into South Padre Island may have occurred by a similar mechanism, but it is also possible that the distribution of S. h. floridana has expanded from the Caribbean Sea northward along the southward western shores of the Gulf of Mexico. Further studies will be necessary to clarify the distribution of S. h. floridana in the Gulf of Mexico. Factors such as human activity, drifting algae or logs may also change the distributions of S. haemastoma and contribute to the intermixing of species in the studied collections.

Abbott (1974) suggested that Stramonita haemastoma canaliculata and S. h. haysae are synonyms. S. h. canaliculata was originally described as "Fusus canaliculatus" from China by Gray (1839) and it was re-identified as the holotype of S. h. canaliculata by Yen (1942). However, S. h. haysae is a new subspecies found in Louisiana, USA (Clench 1927). Based on specimen localities, there is little possibility that S. h. canaliculata and S. h. haysae are the same subspecies. By examining the specimen of S. h. canaliculata and S. h. haysae, Kool (personal communication) suggested that haysae is a large and nodulose adult form of canaliculata. Possibly Abbott (1974) had a similar opinion and therefore he suggested that S. h. canaliculata and S. h. haysae are synonyms. In addition, no other references indicate that S. h. canaliculata has been found in the west Pacific coast or in China. Therefore, it is possible that a mistake in the type-locality of S. h. canaliculata was made by Gray (1839). Consequently, in respecting the need for the uniformity in nomenclature, the older subspecies name canaliculata is suggested for this endemic species as Stramonita canaliculata. In addition, the systematic status of S. h. floridana remains undetermined until further comparison is made with typical S. h. haemastoma from the Mediterranean Sea.

## ACKNOWLEDGEMENTS

We thank Mr. W. Delaune at Louisiana Universities Marine Consortium, Dr. M. Michael and Mr. S. van Arnam in the Whitney Laboratory of the University of Florida, and Dr. Ann Bull in the Louisiana Department of Wildlife and Fisheries for help with the collection of snails for this study. We also thank Mr. P. Rutledge in the Department of Zoology and Physiology for help with statistical analysis of the data, Dr. S. A. Hsu in the Department of Geology and Geophysics for the provision of hydrographic information, Dr. S. W. Matthews in the Department of Botany, Louisiana State University for help with scanning electron microscopy, Dr. S. Kool in the Museum of Comparative Zoology, Harvard University for providing constructive criticism of this manuscript, and Mr. C. Hopkins for help with writing the manuscript.



## LITERATURE CITED

- Abbott, R.T. (1974). American seashells - the marine mollusca of the Atlantic and Pacific coasts of North America. Van Nostrand Reinhold Co., New York.
- Ahmad, M., Skibinski, D.O.F., Beardmore, J.A. (1977). An estimate of the amount of genetic variation in the common mussel Mytilus edulis. Biochem. Genet. 15:833-846.
- Andrews, J. (1971). Sea shells of the Texas coast. University of Texas Press. Austin.
- Awise, J.C. (1975). Systematic value of electrophoretic data. System. Zool. 23:465-481.
- Bandel, K. (1984). The radulae of Caribbean and other Mesogastropoda and Neogastropoda. Zoologische Verhandelingen no. 214. 188pp.
- Barnwell, F.H., Thurman, C.L. II. (1984). Taxonomy and biogeography of the fielder crabs (Ocypodidae: Genus Uca) of the Atlantic and Gulf coasts of eastern North America. Zool. J. Linn. Soc. 81:23-87.
- Bertness, M.D., Yund, P.O., Brown, A.F. (1983). Snail grazing and the abundance of algal crusts on a sheltered New England rocky beach. J. Exp. Mar. Biol. Ecol. 71:147-164.
- Buroker, N.E., Hershberger, W.K., Chew, K.K. (1979). Population genetics of the family Ostreidae. II. Interspecific studies of the genera Crassostrea and Saccostrea. Mar. Biol. 54:171-184.
- Butler, P.A. (1954). The southern oyster drill. Proc. Nat. Shellfish. Assoc. 44:67-75.
- Clench, W.J. (1927). A new subspecies of Thais from Louisiana. The Nautilus 41:6-8.
- Clench, W.J. (1947). The genera Purpura and Thais in the Western Atlantic. Johnsonia 2:61-91.
- Cooke, A.H. (1919). The radula in Thais, Drupa, Morula, Concholepas, Cronia, Iopas and allied genera. Proc. Mala. Soc. London 13:91-110.

- Conrad, T.A. (1837). Descriptions of new marine shells from upper California. J. Acad. Nat. Sci. Phila. 7:265-266. Crothers, J.H. (1983). Variation in dog-whelk shells in relation to wave action and crab predation. Biol. J. Linn. Soc. 20:85-102.
- Dando, P.R., Storey, K.B., Hochachka, P.W., Storey, J.M. (1981). Multiple dehydrogenases in marine molluscs: electrophoretic analysis of alanopine dehydrogenase, strombine dehydrogenase, octopine dehydrogenase and lactate dehydrogenase. Mar. Biol. Lett. 2:249-257.
- D'Asaro, C.N. (1970). Egg capsules of prosobranch mollusks from south Florida and the Bahamas and notes on spawning in the laboratory. Bull. Mar. Sci. 20:414-440.
- Davis, G.M. (1983). Relative roles of molecular genetics, anatomy, morphometrics and ecology in assessing relationships among North American Unionidae (Bivalvia). In: Protein polymorphism: adaptive and taxonomic significance. G.S. Oxford and D. Rollinson eds. Academic Press, London. p. 193-222.
- Etter, R.J. (1989). Life history variation in the intertidal snail Nucella lapillus across a wave-exposure gradient. Ecology 70:1857-1876.
- Ferguson, A. (1980). Biochemical systematics and evolution. John Wiley and Sons, New York, 194pp.
- Gray, J.E. (1839). Molluscos animals, and their shells. In: The Zoology of Captain Beechey's voyage, London, Bohn. p.101-155. plates 33-44.
- Gunter, G. (1979). Studies of the southern oyster borer, Thais haemastoma. Gulf Res. Rept. 6:249-260.
- Harris, H. and D.A. Hopkinson (1978). Handbook of enzyme electrophoresis in human genetics. Supplement. North-Holland Publishing Co., Amsterdam, New York. 299pp.
- Hickman, C.S. (1980). Gastropod radulae and the assessment of form in

- evolutionary paleontology. *Paleobiology* 6:276-294.
- Holt, J., Bartz, M., Lehman, J. (1982). Gulf of Mexico. Regional environmental impact statement. The Minerals Management Service, Imperial Office Building, Metairie, LA 70010. p. 130-158.
- Johnson, C.W. (1934). List of the marine molluscs of the Atlantic coast from Labrador to Texas. *Proc. Boston Soc. Nat. Hist.* XL:118.
- Kool, S.P. (1987). Significance of radular characters in reconstruction of Thaidid phylogeny (Neogastropoda: Muricacea). *The Nautilus* 101:117-132.
- Kool, S.P. (1989). Phylogenetic analysis of the subfamily Thaidinae (Prosobranchia: Neogastropoda: Muricacea). Dissertation. The George Washington University, Washington D.C. pp. 342.
- Kitching, J.A. (1976). Distribution and changes in shell form of Thais spp. (Gastropoda) near Bamfield, B.C. *J. Exp. Mar. Biol. Ecol.* 23:109-126.
- Largen, M.J. (1971). Genetic and environmental influences upon the expression of shell sculpture in the dog whelk (Nucella lapillus). *Proc. Malac. Soc. London* 39:383-388.
- Leipper, D.F. (1954). Physical oceanography of the Gulf of Mexico. In: Gulf of Mexico its origin, waters, and marine life. P.S. Galtsoff ed. Fishery Bulletin of the Fish and Wildlife Service Vol. 55.
- Moore, D.R. (1961). The marine and brackish water Mollusca of the state of Mississippi. *Gulf Res. Rept.* 1:1-58.
- Morris, P.A., Clench, W.J. (1973). A field guide to shells of the Atlantic and Gulf coasts and the West Indies. Houghton Mifflin Co. Boston.
- Murdock, E.A., Ferguson, A., Seed, R. (1975). Geographical variation in Leucine aminopeptidase in Mytilus edulis L. from the Irish coasts. *J. Exp. Mar. Biol. Ecol.* 19:33-41.

- Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89:583-590.
- Palmer, A.R. (1984). Species cohesiveness and genetic control of shell color and form in Thais emarginata (Prosobranchia, Muricacea): preliminary results. *Malacologia* 25:477-491.
- Palmer, A.R. (1985). Quantum changes in gastropod shell morphology need not reflect speciation. *Evolution* 39:699-705.
- Radwin, G.E., Wells, H.W. (1968). Comparative radular morphology and feeding habits of Muricid gastropods from the Gulf of Mexico. *Bull. Mar. Sci.* 18:72-85.
- Richardson, B.J., Baverstock, P.R., Adams, M. (1986). Allozyme electrophoresis: a handbook for animal systematics and population studies. Academic Press, New York, 410pp.
- Roller, R.A., Garton, D.W., Stickle, W.B. (1984). Regeneration of the proboscis, radula and odontophoral cartilage of the southern oyster drill Thais haemastoma canaliculata (Gray) (Prosobranchia: Muricidae) after amputation. *Am. Malac. Bull.* 2:63-73.
- SAS Institute, Inc. (1982). SAS user's guide: statistics. 1982 ed. SAS Institute, Inc., Cary, North Carolina.
- Schaal, B.A., Anderson, W.W. (1974). An outline of techniques for starch gel electrophoresis of enzymes from the American oyster *Crassostrea virginica* Gmelin. Georgia Mar. Sci. Center Tech. Rept. University of Georgia. pp. 17.
- Scheltema, R.S. (1971). Larval dispersal as a means of genetic exchange between geographically separated populations of shallow-water benthic marine gastropods. *Biol. Bull.* 140:284-322.
- Scheltema, R.S. (1972). Dispersal of larvae as a means of genetic exchange between widely separated populations of shoal-water benthic invertebrate species. Fifth European Marine Biological Symposium, 348pp. Piccin Editore, Padua. p. 101-114.
- Scheltema, R.S. (1975). Relationship of larval dispersal, gene-flow

- and natural selection to geographic variation of benthic invertebrates in estuaries and along coastal regions. In: Estuarine Research, Vol. I. Chemistry, biology, and the estuarine system. L.E. Cronin ed. Academic Press, New York. p. 372-391.
- Sieling, F.W. (1960). A notable range extension of the southern drill, Thais haemastoma floridana, into Chincoteague Bay. Chesapeake Science 1:212-215.
- Sneath, P.H.A., Sokal, R.R. (1973). Numerical taxonomy: the principles and practice of numerical classification. W.H. Freeman, San Francisco.
- Spight, T.M. (1977). Latitude, habitat, and hatching type for Muricacean Gastropods. The Nautilus 91:67-71.
- St. Amant, L.S. (1938). Studies on the biology of the Louisiana oyster drill, Thais haemastoma haysae. Master's thesis. Louisiana State University.
- Thorpe, J.P. (1983). Enzyme variation, genetic distance and evolutionary divergence in relation to levels of taxonomic separation. In: Protein polymorphism: adaptive and taxonomic significance. G.S. Oxford and D. Rollinson eds. Academic Press, New York. p. 131-152.
- Walker, R.L. (1982). The gastropod Thais haemastoma in Georgia: T.h. floridana or T.h. canaliculata? Gulf Res. Rept. 7:183-184.
- Williams, A.B., Felder, D.L. (1986). Analysis of stone crabs: Menippe mercenaria (Say), restricted, and a previously unrecognized species described (Decapoda: Xanthidae). Proc. Biol. Soc. Washington 99:517-543.
- Woodruff, D.S., Staub, K.C., Upatham, E.S., Viyanant, V., Yuan, H.-C. (1988). Genetic variation in Oncomelania hupensis: Schistosoma japonicum transmitting snails in China and the Philippines are distinct species. Malacologia 29:347-361.
- Yen, T.-C. (1942). A review of Chinese gastropods in the British

Museum. Proc. Malac. Soc. London 24:220-289.

Appendix 1.1. Buffer systems and stain recipes for oyster drills.

A. Gel size: 20.5 x 19 x 0.6 cm

B. Buffer systems:

1. Tris-citrate gel and electrode buffers pH 8.0, 5 h at 17 v/cm (TC1 8.0)

Tris 625 mM	416.0 g
Citric acid 143 mM	165.0 g
H <sub>2</sub> O	5.5 l

gel buffer working solution  
20:580 with distilled water  
electrode buffer working solution  
straight
2. Tris-citrate buffer pH 7.0, 4 h at 28 v/cm (TC2 7.0)

gel buffer pH 7.0	
Tris 9 mM	1.089 g
Citric acid 2.9 mM	0.609 g
MgCl <sub>2</sub> 0.1 M	10 ml
H <sub>2</sub> O	1 l
electrode buffer pH 7.0	
Tris 135 mM	16.4 g
Citric acid 43 mM	9.03 g
MgCl <sub>2</sub> 0.1 M	10 ml
H <sub>2</sub> O	1 l
3. Tris-citrate buffer pH 7.0, 4 h at 28 v/cm (TC3 7.0)

gel buffer pH 7.0	
Tris 9 mM	1.089 g
Citric acid 2.9 mM	0.609 g
H <sub>2</sub> O	1 l
(add 0.005% NADP (w:v) in gel buffer)	
electrode buffer pH 7.0	
Tris 135 mM	16.4 g
Citric acid 43 mM	9.03 g
H <sub>2</sub> O	1 l
(add 0.005% NADP (w:v) in electrode buffer)	
4. Tris-citrate buffer pH 6.7, 5 h at 22 v/cm (TC4 6.7)

gel buffer pH 6.7	
Tris 256 mM	30.98 g
Citric acid 96 mM	20.16 g
NaOH	1.4 g
H <sub>2</sub> O	1.0 l
working solution	
18:582 with distilled water	
electrode buffer pH 6.3	
Tris 892 mM	216.00 g
Citric acid 344 mM	144.56 g
NaOH	8.4 g
H <sub>2</sub> O	2.0 l
working solution	
200:600 with distilled water	
5. Tris-maleic acid EDTA gel and electrode buffers pH 7.4,  
7 h at 11 v/cm (TM 7.4)

Tris 400 mM	96.8 g
Maleic acid 400 mM	92.8 g
Na <sub>2</sub> EDTA.2H <sub>2</sub> O	29.76 g
MgCl <sub>2</sub> .6H <sub>2</sub> O	16.24 g
NaOH	40.0 g
H <sub>2</sub> O	2.0 l
gel buffer working solution	
15:585 with distilled water	
electrode buffer working solution	
200:600 with distilled water	

6. Phosphate-citrate gel and electrode buffers pH 7.0,  
7 h at 11 v/cm (PC 7.0)
- |  |         |
|--|---------|
| K <sub>2</sub> HPO <sub>4</sub> 210 mM | 73.35 g |
| Citric acid 27 mM                      | 11.39 g |
| H <sub>2</sub> O                       | 2.0 l   |
| gel buffer working solution            |         |
| 15:385 or 24:576 with distilled water  |         |
| electrode buffer working solution      |         |
| straight                               |         |

#### C: Staining recipes

##### ADK

Glucose	100 mg
MgCl <sub>2</sub> 0.1 M	2.1 ml
G-6-PDH	50 I.U.
Hexokinase	80 I.U.
ADP	20 mg
NADP 1% (w:v)	2.5 ml
MTT 1% (w:v)	2.5 ml
PMS 1% (w:v)	0.3 ml
Tris-HCl 0.5 M pH 7.1	10 ml
distilled water	90 ml

##### AlaDH (UV)

L-alanine	70 mg
NADH	2 mg
Pyruvate	6 mg
Tris-HCl 0.1 M pH 7.5	25 ml

(incubate for 30 sec. then discard substrate solution and directly observe under UV light)

##### CK & PK

Glucose	1 g
Hexokinase	0.5 mg
Phosphocreatine	50 mg
ADP	25 mg
NADP 1% (w:v)	1.7 ml
MTT 1% (w:v)	0.6 ml
PMS 1% (w:v)	0.6 ml
MgCl <sub>2</sub> 0.1 M	1.1 ml
Tris-HCl 0.2 M pH 8.0	25 ml
distilled water	25 ml
agar	0.5 g



## EST1 (UV)

4-methylumbelliferyl acetate	10 mg
Acetate buffer pH 5.6	15 ml

presoak in 0.5 M boric acid if gel buffer  
was above pH 7.0

(Acetate buffer:

Sodium acetate	190.5 g
distilled water	7 l

use acetic acid titrate to pH 5.6

## EST2

Fast Blue BB salt	100 mg
substrate solution	3 ml
Tris-HCl 0.5 M pH 7.1	10 ml
distilled water	90 ml

(substrate solution:

na: $\alpha$ -naphthyl acetate	0.5 g
$\beta$ -naphthyl acetate	0.5 g

acetone	50 ml
---------	-------

distilled water	50 ml
-----------------	-------

np: $\alpha$ -naphthyl propionate	0.5 g
-----------------------------------	-------

$\beta$ -naphthyl propionate	0.5 g
------------------------------	-------

acetone	50 ml
---------	-------

distilled water	50 ml
-----------------	-------

## FUM

Fumarate	0.4 g
NAD 1% (w:v)	2 ml
MTT 1% (w:v)	1 ml
PMS 1% (w:v)	0.3 ml
Malate dehydrogenase	100 I.U.
Tris-HCl 0.2 M pH 8.0	100 ml

## GDH

Glutamate	100 mg
NADP 1% (w:v)	0.5 ml
NAD 1% (w:v)	0.5 ml
ADP	5 mg
MTT 1% (w:v)	0.5 ml
PMS 1% (w:v)	0.1 ml
Tris-HCl 0.5 M pH 7.0	15 ml

## GOT1 &amp; 2

Fast Blue BB salt	100 mg
$\alpha$ -ketoglutaric acid	37 mg
L-aspartic acid	133 mg
Na <sub>2</sub> EDTA	50 mg
Polyvinyl Pyrrolidone	0.5 g
Na <sub>2</sub> HPO <sub>4</sub>	1.42 g
distilled water	100 ml

## GPT1 &amp; 2 (UV)

L-alanine	250 mg
$\alpha$ -ketoglutaric acid	146 mg
NADH	20 mg
Lactate dehydrogenase	27.5 I.U.
Tris-HCl 0.1 M pH 7.5	20 ml

(incubate for 30 sec. then discard substrate solution  
and directly observe under UV light)

#### LAP1 & 2

L-leucine 4-methoxy- $\beta$ -naphthylamide (LAP1) or	
L-leucyl- $\beta$ -naphthylamide diHCl (LAP2)	25 mg
Tris-malate buffer 0.2 M pH 6.4	50 ml
(incubate 45min.) then stain with	
Fast black K	50 mg
Tris-malate buffer 0.2 M pH 6.4	50 ml
Tris-malate buffer	
Tris 200 mM	24.2 g
H <sub>2</sub> O	1 l
(adjust the pH to 6.4 by adding	
Maleic acid into the buffer)	

#### MDH

Malic acid 2.0 M pH 7.0	6 ml
NAD 1% (w:v)	2.5 ml
NBT 1% (w:v)	2.5 ml
PMS 1% (w:v)	0.6 ml
Tris-HCl 0.2 M pH 8.0	40 ml

#### LeuAla

Peptides (Leucyl-alanine)	20 mg
Peroxidase	10 mg
O-dianisidine diHCl	10 mg
snake venom	10 mg
MnCl <sub>2</sub> 0.25 M	0.5 ml
Tris-HCl 0.2 M pH 8.0	25 ml
Tris-HCl 0.2 M pH 8.0	25 ml
agar	0.5 g

#### PGM

G-1-P	85 mg
G-6-PDH	40 I.U.
MgCl <sub>2</sub> 0.1 M	5 ml
NADP 1% (w:v)	0.5 ml
PMS 1% (w:v)	0.5 ml
MTT 1% (w:v)	1 ml
Tris-HCl 0.2 M pH 8.0	5 ml
distilled water	30 ml

#### 6-PGDH

6-Phosphogluconic acid	20 mg
NADP 1% (w:v)	0.1 ml
PMS 1% (w:v)	0.1 ml
MTT 1% (w:v)	0.4 ml
MgCl <sub>2</sub> 0.1 M	7 ml
Tris-HCl 0.2 M pH 8.0	10 ml

(stain in dark at room temperature,  
place kimwipe over surface of gel  
then pour on stain)

#### XDH

Hypoxanthine	100 mg
--------------	--------

Tris-HCl 0.2 M pH 8.0	100 ml
(heat above ingredients- don't boil then cool to add other ingredients)	
NAD 1% (w:v)	2 ml
MTT 1% (w:v)	1 ml
PMS 1% (w:v)	0.3 ml

Appendix 1.2 Mean allelic frequencies of Stramonita canaliculata, S. haemastoma floridana, and S. rustica.  
(RM: relative allelic mobility; N: sample size)

Locus	Allele (RM)	<u>S. canaliculata</u>	<u>S. h. floridana</u>	<u>S. rustica</u>
<u>Adk</u>	N	712	188	5
	200	0	0	0.400
	120	0.003	0.010	0
	100	0.992	0.731	0.600
	79	0.005	0.243	0
	43	0	0.016	0
<u>Aladh</u>	N	741	221	5
	167	0.001	0	0
	153	0.021	0	0
	133	0.021	0.009	0
	117	0	0.001	0.100
	100	0.935	0.961	0.100
	87	0.001	0.029	0.800
	73	0.029	0	0
<u>Est1</u>	N	723	221	5
	140	0	0.001	0
	122	0.002	0.010	0
	100	0.998	0.986	1.000
	89	0	0.003	0
<u>Est2</u>	N	741	221	5
	107	0.001	0	0
	100	0.999	1.000	1.000
<u>Fum</u>	N	741	221	5
	100	0.996	0.039	0
	85	0.004	0.961	1.000
<u>Gdh</u>	N	590	221	5
	122	0.001	0.032	0.600
	100	0.999	0.968	0.400
<u>Got2</u>	N	740	221	5
	120	0.008	0.001	0
	100	0.992	0.983	0
	no activity	0	0.016	1.000
<u>Gpt1</u>	N	736	221	5
	100	1.000	1.000	1.000
<u>Gpt2</u>	N	741	221	5
	125	0	0.005	0.300
	108	0.025	0.019	0.600
	100	0.973	0.934	0.100
	83	0.002	0.042	0

<u>Lap1</u>	N	529	221	5
	162	0.001	0.005	0
	150	0.024	0.073	0.100
	131	0.013	0.011	0
	125	0.311	0.538	0.300
	110	0.019	0.014	0.200
	100	0.401	0.007	0.300
	90	0.024	0.101	0.100
	84	0.153	0.179	0
	56	0.039	0.009	0
	42	0.014	0	0
<u>LeuAla</u>	N	741	221	5
	117	0.006	0.006	0.100
	111	0.001	0	0
	100	0.980	0.032	0.300
	88	0.010	0.876	0.500
	75	0.003	0.085	0.100
	64	0	0.001	0
<u>Mdh</u>	N	741	220	5
	135	0.001	0	0
	100	0.992	0.041	0
	67	0.007	0.959	1.000
<u>Pgm</u>	N	739	221	5
	130	0.001	0.011	0
	115	0.025	0.025	0.100
	100	0.974	0.959	0.900
	96	0	0.005	0
<u>Pk</u>	N	597	221	5
	100	1.000	0.998	1.000
	78	0	0.001	0
6- <u>Pgdh</u>	N	612	219	5
	200	0	0	0.400
	178	0	0.002	0
	162	0.013	0	0
	111	0	0.008	0
	100	0.977	0.071	0
	88	0.005	0.003	0.600
	81	0.004	0.607	0
	63	0.001	0.309	0
<u>Xdh</u>	N	728	221	5
	100	1.000	1.000	1.000

---

CHAPTER TWO  
ADAPTATION TO SALINITY IN STRAMONITA "THAIS" CANALICULATA AND S.  
HAEMASTOMA FLORIDANA : PATTERNS OF PHENOTYPIC AND  
GENOTYPIC VARIATIONS

ABSTRACT

Six populations of the southern oyster drill Stramonita canaliculata and three populations of S. haemastoma floridana, along the Gulf of Mexico and on the east coast of Florida, representing low to high salinity habitats, have been sampled to determine if patterns of phenotypic and genotypic variation correlate with salinity clines. The 28-day high-salinity LC-50 is lower in four populations of S. canaliculata (44.4 to 51.5 ‰ S) than in the population of S. haemastoma floridana (54.8 ‰ S). Low-salinity tolerance limits in S. canaliculata are lower (3.5 to 7.1 ‰ S) than in S. h. floridana (7.3 ‰ S). In S. canaliculata, snails from medium to high salinity areas exhibit a higher high-salinity LC-50 and a shorter period to reach a stable high-salinity tolerance limit than populations from low to medium salinity areas. In contrast, the low-salinity LC-50 exhibits the opposite trend. S. canaliculata and S. h. floridana have different allelic frequencies at Fum, Lap1, Lap2, LeuAla, Mdh, and 6-Pgdh loci. The percentage of loci polymorphic, of the 17 enzyme loci assayed, is 5.9 - 11.8 in S. canaliculata and 35.3 - 41.2 in S. h. floridana, and mean individual heterozygosity differs significantly between S. canaliculata and S. h. floridana (0.04 - 0.07 vs 0.09 - 0.16). Little genetic variation has been found among populations within the two species and no salinity-related cline was observed in those salinity-related enzyme loci. Their protein-oriented metabolism and avoidance behavior to rapid salinity changes in oyster drills may explain the insignificant allozymic adaptation to salinity.

INTRODUCTION

Physiological responses of organisms to environmental changes may not involve changes in their genetic constitution. However, considerable evidence indicates that adaptation to environmental changes

in organisms is partially due to their genotypes. For example, the scope for growth of Stramonita "Thais" haemastoma (now S. canaliculata) exhibits a significant positive relationship with total heterozygosity of six polymorphic loci at 7.5, 20 and 35 ‰ S (Garton 1984). The allelic frequencies of phosphoglucose isomerase (Pgi) and phosphoglucomutase (Pgm) vary with thermal history (Nevo et al. 1977, Hoffmann 1983, Watt 1983) or heavy metal pollution (Lavie and Nevo 1982). The selection of alleles of aminopeptidase-I (Lap) (Koehn and Hilbish 1987, Beaumont et al. 1988, McDonald and Siebenaller 1989, Garthwaite 1986, Buroker 1983), or glutamate-pyruvate transaminase (Gpt) (Burton 1983, Burton and Feldman 1983) is related to environmental salinities.

The southern oyster drills, Stramonita "Thais" canaliculata and S. haemastoma floridana are widely distributed estuarine gastropods. Although the low-salinity distribution limit of S. canaliculata and S. h. floridana in nature is 15 ‰ S (St. Amant 1957), they have survived for weeks at salinities of 3.5 - 7.5 ‰ S in the laboratory (Garton and Stickle 1980, Hildreth and Stickle 1980, Schechter 1943). Also, they can withstand fresh water for several days without apparent harm (Butler 1954, Gunter 1979). In addition, it has been reported that in natural environments drills withstood 5 ‰ S, or in almost fresh water, for about 6 weeks (Butler 1954). However, their distribution is limited by extended periods of exposure to low salinity environments (Gunter 1979).

Although studies on nitrogen metabolism and bioenergetic responses along salinity gradients on the southern oyster drills have been investigated extensively (Stickle and Howey 1975, Hildreth and Stickle 1980, Garton and Stickle 1980, Findley et al. 1978, Garton 1984, Garton and Stickle 1985, Kapper et al. 1985, Stickle 1985), little is known about the relative role of environmental and genetic effects on their salinity adaptation.

Like other molluscs, Stramonita canaliculata is an osmoconformer

and adjusts its intracellular osmotic pressure, in part, by altering the concentration of free amino acids (primarily alanine and glycine) to adapt to environmental salinity changes (Kapper et al. 1985). These amino acids are derived from multiple metabolic pathways (Bishop et al. 1981, Deaton et al. 1984, Pierce 1982, Shumway and Gabbott 1977, Zurburg and Zwaan 1981, Kapper et al. 1985). In gastropods, the enzymes which are directly or indirectly involved in this metabolism may be alanopine dehydrogenase (AlaDH) (Livingstone et al. 1983), aminopeptidases (i.e. LAP and leucine-alanine di-aminopeptidase (LeuAla)), glutamate dehydrogenase (GDH), glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), pyruvate kinase (PK) (Bishop et al. 1981, 1983, Wickes and Morgan 1976), and others (Fig. 2.1). Using electrophoresis, genetic variation to salinity adaptation can be determined by estimating the variation in the allelic frequencies of these enzyme loci among populations from different salinity habitats.

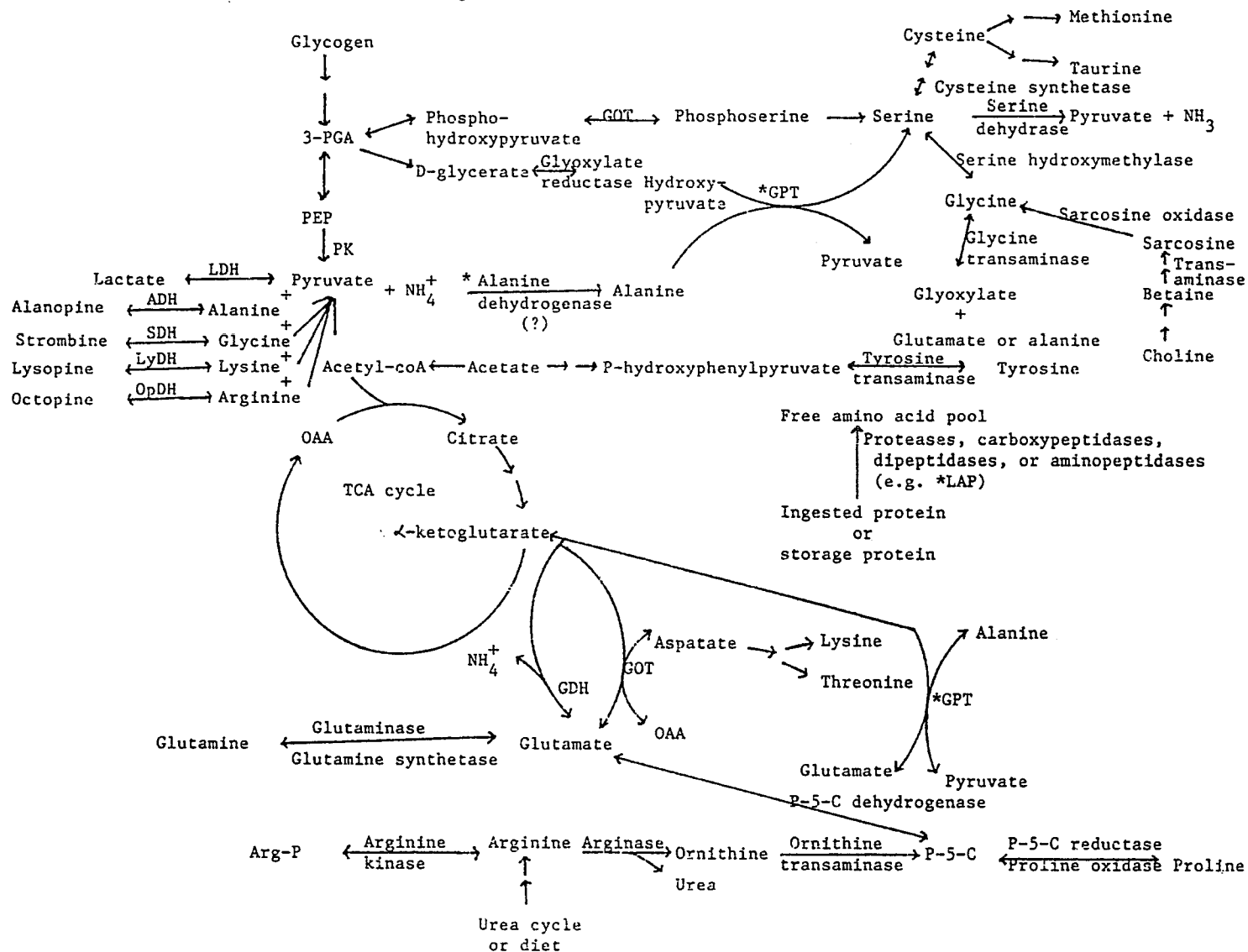
In this study, salinity-related phenotypic and genotypic variations were investigated. Consequently, the contributions of environmental and genetic factors to salinity adaptation were estimated. Experiments were conducted (1) to determine salinity tolerance (i.e. phenotypic variation) among populations and between species of Stramonita canaliculata and S. haemastoma floridana from high and low salinity habitats and (2) to determine the genetic variations in enzyme loci which are directly or indirectly involved in amino acid metabolism in S. canaliculata and S. h. floridana.

Although Stramonita haemastoma canaliculata and S. h. floridana have been identified as subspecies of S. haemastoma (Abbott 1974, Andrews 1971, Clench 1947) around Florida and the Gulf of Mexico, recent electrophoretic studies indicate that S. h. canaliculata and S. h. floridana are separate species (see Chapter 1). Also, the genus, Thais, has been revised to Stramonita based on anatomy and radular characteristics (Kool 1987). Therefore, Stramonita canaliculata and S.



Fig. 2.1 Summary of pathways leading to accumulation of amino acids.

### Summary of pathways leading to accumulation of amino acids



h. floridana are treated as separate species in this study. The genetic variation was only determined by the major species of the collection without any possible hybrids involved. Because the salinity tolerance study was accomplished before the dynamics of the subspecies complex was understood, the salinity tolerance experiments in Caminada Pass and Marineland collections might have a potential bias of less than 8% due to possible hybrids or a mixture of the two species of Stramonita.

#### MATERIALS AND METHODS

##### Snails

Snails were collected around the Gulf of Mexico and on the east coast of Florida in the summer and autumn months of 1987-1989 (Fig. 2.2). Six populations of Stramonita canaliculata were collected from high to low salinity habitats (Table 2.1), i.e. Red Fish Bay, TX, Caminada Pass, LA, Manila, LA, Henderson Point, MS, Ship Island, MS, and Pensacola Bay, FL. Three populations of S. haemastoma floridana were collected from South Padre Island, TX, Ponce Inlet, FL and Marineland, FL.

##### Salinity tolerance experiments

The salinity tolerance limits (LC-50) were determined in order to address the salinity-related phenotypic variation in snails from Red Fish Bay, Caminada Pass, Manila, Pensacola Bay, and Marineland. Snails were collected and maintained in aquaria with artificial seawater of ambient salinity for three days prior to salinity transfers. Afterwards, a stepwise salinity transfer schedule was initiated at the rate of 2 ‰ S per day from their ambient salinity to the final experimental salinities. The experimental salinities were 5, 10, 15, 20, 30, 40, 45, 50, 55, 60, and 65 ‰. The initial sample size (=n) in each salinity treatment was 20 snails. By using the Spearman-Kärber method (Finney 1971), LC-50 values were calculated from the daily mortality data in each salinity treatment for a 28 day period after reaching final experimental salinity.

Fig. 2.2 Collection sites for the southern oyster drills, Stramonita canaliculata and S. haemastoma floridana, on the Gulf of Mexico and the east coast of Florida. 1. South Padre Island (SP); 2. Red Fish Bay (RF); 3. Caminada Pass (CP); 4. Manila (MA); 5. Henderson Point (HP); 6. Ship Island (SI); 7. Pensacola Bay (PB); 8. Ponce Inlet (PI); 9. Marineland (ML)

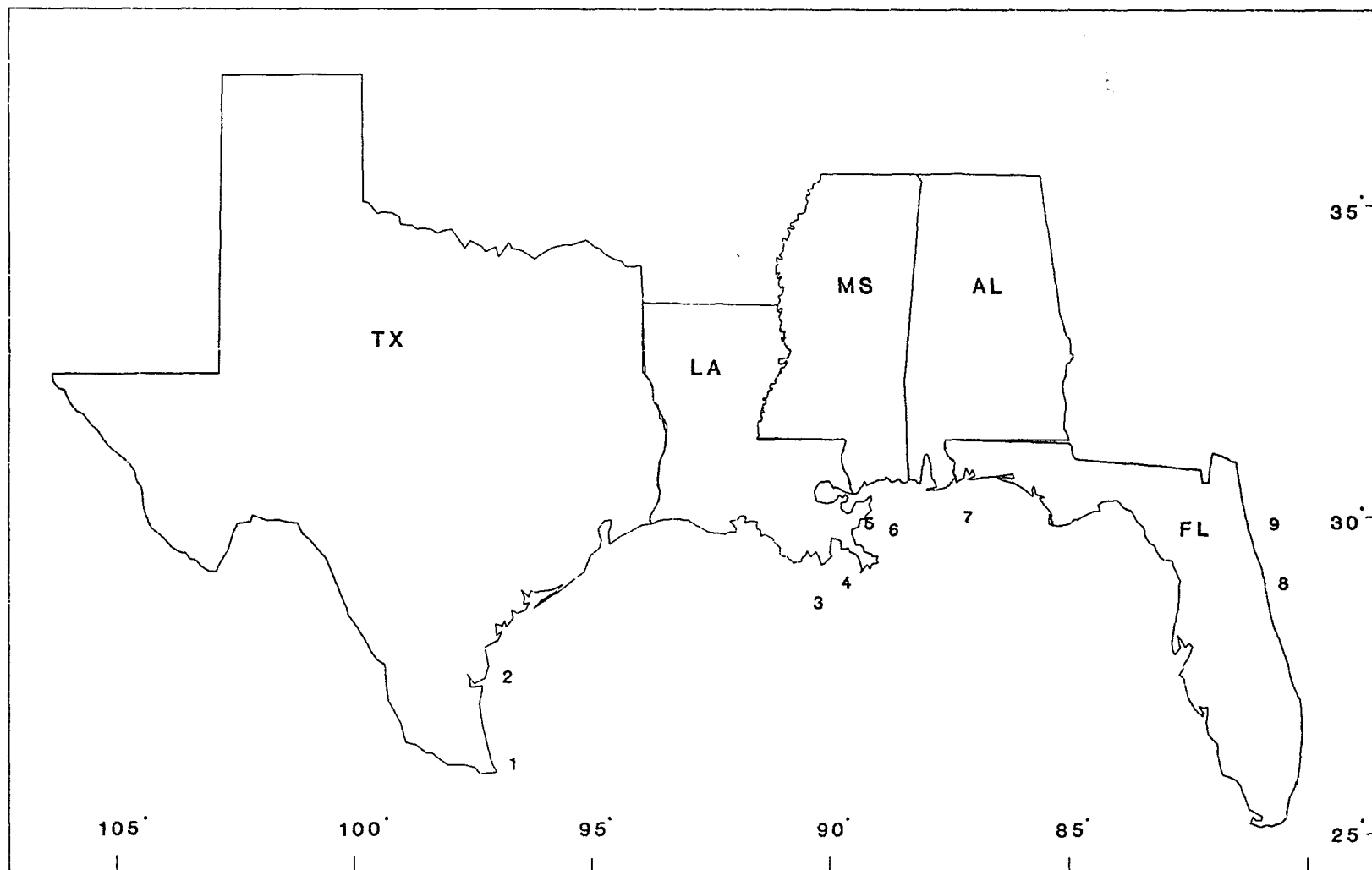


Table 2.1 Stramonita canaliculata and S. haemastoma floridana collection sites and local salinity data.

Location	Abb.	Time of collection	Salinity on the collection date (‰/ooS)	Monthly salinity range (‰/ooS)	Year	Citation
<u>Stramonita canaliculata</u>						
Red Fish Bay, TX	(RF)	July, 1989	42	23 - 40	1958 - 1959	Hedgpeth, 1967
Caminada Pass, LA	(CP)	October, 1989	32	19 - 31	1968 - 1969	Barrett, 1971
Manila, LA	(MA)	November, 1989	17	5 - 23	1969	Barrett, 1971
Henderson Point, MS	(HP)	October, 1989	18	2 - 27	1986 - 1987	Demoran, 1988
Ship Island, MS	(SI)	October, 1989	31			
Pensacola Bay, FL	(PB)	June, 1988	30	17 - 30	1962 - 1963	Cooley, 1978
<u>Stramonita haemastoma floridana</u>						
South Padre Island, TX	(SP)	July, 1989	38			
Ponce Inlet, FL	(PI)	August, 1989	38			
Marineland, FL	(ML)	August, 1989	37			

### Electrophoretic studies

Electrophoresis was used to determine salinity-related allozyme variation and genetic structure of nine populations (Table 2.1). Snails were kept in recirculating artificial seawater of ambient salinity for three days to eliminate artifactual electrophoretic patterns due to gut contents. The snails were frozen at  $-70^{\circ}\text{C}$  until dissected. Foot muscle or whole soft tissue was used. The eighteen enzymes assayed by horizontal starch gel electrophoresis are: adenylate kinase (ADK), alanopine dehydrogenase (AlaDH), esterase 1 and 2 (EST1 and EST2), fumarase (FUM), glutamate dehydrogenase (GDH), glutamate-oxaloacetate transaminase 1 and 2 (GOT1 and GOT2), glutamate-pyruvate transaminase 1 and 2 (GPT1 and GPT2), leucine aminopeptidase 1 and 2 (LAP1 and LAP2), di-peptidase specific to the substrate of Leucine-alanine (LeuAla), malate dehydrogenase (MDH), phosphoglucumutase (PGM), pyruvate kinase (PK), 6-phosphogluconate dehydrogenase (6-PGDH), and xanthine dehydrogenase (XDH). The procedures for sample preparation and the methods used for electrophoresis were as in Appendix 1.1.

The mean number of alleles per locus, the percentage of loci polymorphic, and the mean individual heterozygosity were calculated for each population. Because of inconsistent interpretation of Lap2 allozyme in Stramonita canaliculata and of Got2 in S. haemastoma floridana, these loci were not included in the analysis. Wright's hierarchical F-statistics (Table 2.2) (Wright 1978, Weir and Cockerham 1984) were calculated for each locus and population to determine genotypic variation of Stramonita canaliculata and S. haemastoma floridana. The measure of the genetic variation in the total population is additively composed of the measures of genetic variation within and between subpopulations (Wright 1978). If, in a population, all subpopulations (deme) under study have the same pattern of variation, then variation between demes is absent ( $F=0$ ). The range of 0.15 to 0.25 indicates moderately great differentiation. And a F value above

Table 2.2 Summary of the variance table for Wright's hierarchical F-statistics.

Source of variation	Variance component	F-statistics
Region within total	$\sigma^2_{RT}$	$F_{RT} = \sigma^2_{RT} / \sigma^2_{GT}$
Population within region	$\sigma^2_{PR}$	$F_{PR} = \sigma^2_{PR} / (\sigma^2_{GT} - \sigma^2_{RT})$
Deme within population	$\sigma^2_{DP}$	$F_{DP} = \sigma^2_{DP} / (\sigma^2_{GT} - \sigma^2_{RT} - \sigma^2_{PR})$
Individual within deme	$\sigma^2_{ID}$	$F_{ID} = \sigma^2_{ID} / (\sigma^2_{ID} + \sigma^2_{GI})$
Genes within individuals	$\sigma^2_{GI}$	
Total	$\sigma^2_{GT}$	



0.25 is considered as a great differentiation between demes. In this study, the four levels of Wright's  $F$  statistics used were  $F_{ID}$ : individuals within demes (populations) (as a measure of departure of genotypic frequencies from Hardy-Weinberg expectations),  $F_{DP}$ : demes within population (populations in a state),  $F_{PR}$ : populations within region (populations in eastern or western states), and  $F_{RT}$ : regions within total area sampled. All of the analyses were calculated by SAS programming (SAS Institute, Inc. 1982).

## RESULTS

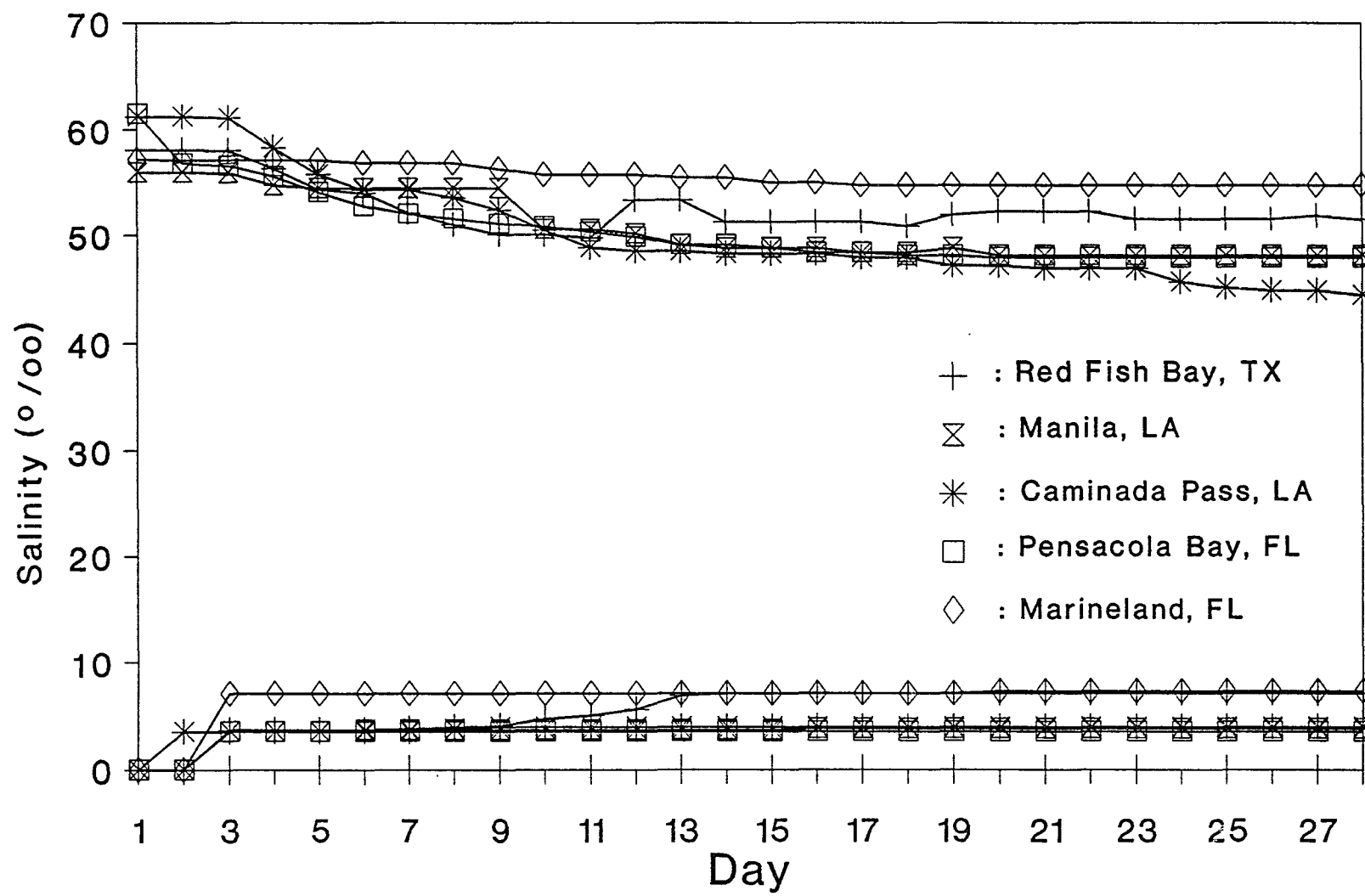
The 28-day salinity tolerance data indicate that populations of Stramonita canaliculata have a high-salinity tolerance limit (44.4 to 51.5 ‰ S) which is lower than S. haemastoma floridana (54.8 ‰ S) (Table 2.3 and Fig. 2.3). In contrast, S. canaliculata is more tolerant of low salinity than S. h. floridana. In S. canaliculata, the 28-day high-salinity LC-50 is higher (51.48 ‰ S) with a shorter period to reach a stable high-salinity tolerance limit (7 days) in the Red Fish Bay population than in other populations (44.4 to 48.1 ‰ S; 14 days) (Table 2.3). Populations from low to medium salinity habitats (i.e. Caminada Pass, Manila, and Pensacola Bay) have a lower low-salinity tolerance limit (3.5 to 3.9 ‰ S) with a shorter period to reach a stable low-salinity tolerance limit (3 days) than the population from the medium to high salinity area (i.e. Red Fish Bay with 7.1 ‰ S; 13 days) (Table 2.3 and Fig. 2.3).

Stramonita canaliculata and S. haemastoma floridana have different allelic frequencies at the Fum, Lap1, Lap2, LeuAla, Mdh, and 6-Pgdh loci. Detailed allelic frequencies of S. canaliculata and S. h. floridana are shown in Appendix 2.1. Within each locus and population of S. canaliculata, genotypic frequencies are in agreement with Hardy-Weinberg expectations except Pgm at Caminada Pass and Ship Island, 6-Pgdh at Henderson Point and Ship Island, and LeuAla at Red Fish Bay (Appendix 2.1). This observation is also indicated by higher values

Table 2.3 28-day LC-50 values and 95% confidence intervals are given for 5 collections of Stramonita as a function of duration of exposure to experimental salinities.

Species		<u>Stramonita canaliculata</u>								<u>S. haemastoma floridana</u>	
Location		Red Fish Bay		Caminada Pass		Manila		Pensacola Bay		Marineland	
Days of exposure		upper limit	lower limit	upper limit	lower limit	upper limit	lower limit	upper limit	lower limit	upper limit	lower limit
7		52.5±3.0	3.8±0.4	54.4±1.1	3.5± 0	54.5± 0	3.7±0.2	52.1±1.1	3.5± 0	56.9±0.7	7.1± 0
14		51.3±1.2	7.1± 0	48.3±1.3	3.9±0.4	48.8±1.6	3.7±0.2	49.1±1.2	3.5± 0	55.5±1.3	7.1± 0
21		52.3±0.4	7.1± 0	46.9±1.3	3.9±0.4	48.1±1.8	3.8±0.4	47.9±0.9	3.5± 0	54.8±1.4	7.3±0.4
28		51.5±0.9	7.1± 0	44.4±1.4	3.9±0.4	48.1±1.8	3.8±0.4	47.9±0.9	3.5± 0	54.8±1.4	7.3±0.4

Fig. 2.3 The 28-day LC-50 of Stramonita canaliculata and S. haemastoma floridana.



(e.g. LeuAla  $F_{ID} = 0.15$ ) in the Wright's F statistics (Table 2.4). Similarly, in three populations of S. h. floridana, no significant departure from Hardy-Weinberg expectations is detected as is indicated by the similarity in observed and expected heterozygosity except in the Adk locus at Marineland, Gdh at Ponce Inlet, and Lap1 and Pgm at South Padre Island (Appendix 2.1). These departures indicate slight heterozygote deficiencies. The mean numbers of alleles per locus is similar in S. canaliculata and S. h. floridana. However, the percentage of polymorphism is three times higher in S. h. floridana (Table 2.5). Also, the mean individual heterozygosity is significantly higher in S. h. floridana than in S. canaliculata.

Salinity-related genotypic variation at enzyme loci of Adh, Gdh, Got1, Got2, Gpt1, Gpt2, Lap1, Lap2, LeuAla, and Pk among populations of Stramonita canaliculata and S. haemastoma floridana was also determined by the F statistics:  $F_{DP}$ ,  $F_{PR}$ , and  $F_{RT}$ . With the exception of the Gdh locus in S. h. floridana, little genetic variation was found within the two species as is indicated by F values being insignificantly different from zero (Table 2.4). However, the F statistics for the Gdh locus may be inflated by the small sample size ( $n=19$ ) and rare genotypes found in the Ponce Inlet population (Appendix 2.1). Although S. canaliculata was collected from different salinity habitats, there are no salinity-related clines detected in any of the ten enzyme loci (Table 2.4). The amount of interpopulation variability in other enzyme loci (i.e. Adk, Est1, Est2, Mdh, Pgm, and 6-Pgdh) also exhibits insignificant differentiation among the six populations of S. canaliculata and three populations of S. h. floridana.

#### DISCUSSION

The 28-day high-salinity LC-50 values are lower in Stramonita canaliculata than in S. haemastoma floridana. In S. canaliculata, the 28-day low-salinity LC-50 is similar to the 5 ‰ S value reported by Stickle (1985). And, the low-salinity LC-50 in S. h. floridana is

Table 2.4 Wright's hierarchical F-statistics of Stramonita canaliculata and S. haemastoma floridana. (n= locus not scored, m= monomorphic locus)

Species	<u>Stramonita canaliculata</u>				<u>S. h. floridana</u>		
Locus	F <sub>ID</sub>	F <sub>DP</sub>	F <sub>PR</sub>	F <sub>RT</sub>	F <sub>ID</sub>	F <sub>DP</sub>	F <sub>RT</sub>
<u>Adk</u>	-0.004	0.005	-0.004	-0.001	0.240	0.085	-0.067
<u>Aladh</u>	0.012	0.003	-0.003	0	-0.019	-0.002	0.010
<u>Est1</u>	-0.002	-0.004	0.007	-0.003	-0.012	0.001	-0.001
<u>Est2</u>	-0.002	0.005	-0.002	-0.003	m	m	m
<u>Gdh</u>	0.001	0	-0.001	-0.001	0.447	0.283	-0.325
<u>Got1</u>	-0.004	0.006	-0.005	0.001	n	n	n
<u>Got2</u>	-0.008	0.004	-0.001	-0.004	0.003	-0.011	0.007
<u>Gpt2</u>	-0.029	0	0.002	0.005	-0.052	0.057	-0.025
<u>Lap1</u>	0.024	0	-0.001	-0.002	0.111	-0.008	0.008
<u>Lap2</u>	n	n	n	n	0.044	-0.012	0.006
<u>LeuAla</u>	0.150	0	-0.002	0.006	-0.033	0.005	0.004
<u>Mdh</u>	-0.002	0.005	-0.002	-0.003	m	m	m
<u>Pgm</u>	0.198	0.010	-0.002	0.001	0.172	-0.002	-0.003
<u>6-Pgdh</u>	0.199	-0.001	-0.001	-0.002	-0.046	0.012	0.015
Mean	0.021	0	-0.001	-0.002	0.077	0.022	-0.009
S.E.	0.028	0.002	0	0.002	0.052	0.020	0.017

Table 2.5 Summary of genetic variations in Stramonita canaliculata and S. haemastoma floridana.

Population	Number of loci	Mean number alleles per locus $\pm$ S.E.	Percent polymorphic loci (.95 criterion)	Mean heterozygosity $\pm$ S.E. observed      expected	
<hr/>					
<u>Stramonita canaliculata</u>					
CP	17	2.53 $\pm$ .49	11.8	.042 $\pm$ .004	.065 $\pm$ .009
HP	17	2.24 $\pm$ .50	11.8	.059 $\pm$ .006	.064 $\pm$ .010
MA	17	1.88 $\pm$ .49	5.9	.053 $\pm$ .006	.051 $\pm$ .008
PB	17	2.18 $\pm$ .48	11.8	.067 $\pm$ .006	.063 $\pm$ .009
RF	17	2.35 $\pm$ .51	11.8	.040 $\pm$ .004	.066 $\pm$ .009
SI	17	2.35 $\pm$ .54	11.8	.061 $\pm$ .006	.065 $\pm$ .011
<hr/>					
<u>Stramonita haemastoma floridana</u>					
ML	17	2.53 $\pm$ .45	35.3	.092 $\pm$ .007	.105 $\pm$ .015
SP	17	2.35 $\pm$ .41	35.3	.118 $\pm$ .009	.128 $\pm$ .017
PI	17	2.12 $\pm$ .38	41.2	.155 $\pm$ .020	.154 $\pm$ .035

similar to the range of 3.5 to 7 ‰ S reported by Schechter (1943) for a shorter exposure period. The general trend for the salinity tolerance studies is that snails from medium to high salinity areas exhibit a higher high-salinity tolerance limit with a shorter period to reach stable high-salinity tolerance limit than snails from low to medium salinity areas. In contrast, the low-salinity LC-50 values exhibit the opposite trend.

Three of the ten salinity-related enzyme loci (i.e. Lap1, Lap2, and LeuAla) have different predominant alleles in Stramonita canaliculata and S. haemastoma floridana (Appendix 2.1). Slight differences in salinity tolerance between the two euryhaline species may, in part, be due to the differences in their genetic structure.

The response to osmotic stress involves many enzymes; however, except for LAP and GPT, little information is available on these enzymes. Like the Lap and Gpt loci, other salinity-related enzyme loci also exhibit little genetic variation among populations of either species studied, even though these oyster drills are from habitats of different salinity habitats and exhibit different salinity tolerances. In other words, salinity adaptation in these two species of Stramonita is primarily due to environmental effects.

In the past ten years, a number of investigations on enzyme polymorphism suggest that the levels and patterns of enzyme diversity vary nonrandomly within and between species and are often associated with environmental factors. For example, the allelic frequencies in Pep-2 and Pgm (substrate= glycyl-leucine) varied with geographic clines in the dogwhelk, Nucella lamellosa (Grant and Utter 1988). The correlation of enzyme polymorphism in Lap with environmental salinity has been extensively studied in the blue mussel, Mytilus edulis (Koehn et al. 1980a, McDonald and Siebenaller 1989, Gartner-Kepkay and Zouros 1983), the mussel M. trossulus (McDonald and Siebenaller 1989), the bivalve Geukensia demissa (Garthwaite 1986), and the oyster Crassostrea



virginica (Buroker 1983). In laboratory studies, selection of certain Lap genotypes in M. edulis occurs during the post-larval to juvenile stage (Beaumont et al. 1989). The correlation of Gpt polymorphism with salinity is also reported in the copepod Tigriopus californicus (Burton and Feldman 1983). In addition, Lap polymorphism is also correlated with the mean temperature in Crassostrea virginica (Rose 1984); or with the combination of temperature and salinity in the snail Tegula funebris (Byers 1983). However, no significant correlation of Lap polymorphism with environmental factors is reported in Geukensia demissa from San Francisco Bay (Garthwaite 1986).

When Mytilus edulis are subjected to salinity transfer from 30 to 15 ‰ S, greater rates of nitrogen loss and tissue weight loss are observed in those individuals which have certain Lap genotypes, e.g. Lap<sup>94/94</sup> with its higher efficiency of regulation of its free amino acid pool (Hilbish and Koehn 1985, Moore et al. 1980, Hilbish et al. 1982). Consequently, the depletion of nitrogen resources results in a genotype-dependent mortality during the autumn months when the food supply is low (Koehn et al. 1980b). Also, similar studies of nitrogen metabolism have been correlated with the Lap locus in the clam Mercenaria mercenaria (Koehn et al. 1980a), in the southern oyster drill Stramonita canaliculata (Garton and Berg 1989) and in the Gpt locus in Tigriopus californicus (Burton and Feldman 1983). The efficiency of nutrient source usage in the land snail Cepaea nemoralis (Brussard 1974) and of fecundity in the oyster drill Urosalpinx cinerea are Lap genotype-dependent (Cole 1979). Commonly, in these studies, the organisms which have been used were either sessile organisms, i.e. Mytilus edulis, M. trossulus, and Crassostrea virginica or herbivores, i.e. Geukensia demissa, Tegula funebris, Cepaea nemoralis, and Tigriopus californicus. The exception to this observation is Urosalpinx cinerea, a carnivore.

Although it has been shown that the rate of free amino acid pool

change during salinity adaptation is related to genotypic differences at the Lap or Gpt locus (Hilbish and Koehn 1985, Burton and Feldman 1983), it is not necessarily true that the depletion of nitrogen resources will occur in all the species. Oyster drills, including Stramonita canaliculata, S. haemastoma floridana and Urosalpinx cinerea are carnivorous; therefore, protein resources are much more available to them than to filter feeders or algal scrappers. Oxygen : nitrogen ratios indicate that S. canaliculata has a protein-oriented metabolism (Stickle 1985). However, bivalves such as M. edulis primarily catabolize carbohydrate or lipid, except in the gamete reabsorption phase of the reproduction cycle, when protein is the primary metabolic substrate (Widdows 1978a, b). In addition, adult oyster drills can easily crawl as far as 9 m/h on hard surfaces or on very soft bottoms (Butler 1954, St. Amant 1957). When large amplitudes of salinity variation occur, oyster drills may close their operculum and switch to partial anaerobiosis (see chapter 3) or move into deeper water channels with higher salinity seawater (Butler 1954). Consequently, the abundance of nitrogen provided by their protein-oriented metabolism and avoidance behavior to rapid salinity changes in oyster drills may diminish the necessity of genotypic adaptation at salinity-related enzyme loci to salinity.

## ACKNOWLEDGEMENTS

We thank Mr. W. Delaune at Louisiana Universities Marine Consortium Foundation, Inc. and Mr. J. Guerin for the collection of snails for this study. We also thank Mr. P. Rutledge in the Department of Zoology and Physiology for help with analysis of the data, Dr. W. Demoran for provision of salinity data by the support of 88-309 Project 2-296-R, Fisheries Assessment and Monitoring, National Marine Fisheries Service, and Mr. C. Hopkins for help with writing the manuscript.

## LITERATURE CITED

- Abbott, R.T. (1974). American seashells - the marine mollusca of the Atlantic and Pacific coasts of North America. Van Nostrand Reinhold Co., New York.
- Andrews, J. (1971). Sea shells of the Texas coast. University of Texas Press. Austin.
- Barrett, B. B. (1971). Cooperative Gulf of Mexico estuarine inventory and study, Louisiana. Phase II, Hydrology and Phase III, Sedimentology. Louisiana Wild Life and Fisheries Commission, New Orleans, Louisiana. 191pp.
- Beaumont, A.R., Beveridge, C.M., Barnet, E.A., Budd, M.D., Smith-Chamosa, M. (1988). Genetic studies of laboratory reared Mytilus edulis. I. genotype specific selection in relation to salinity. Heredity, 61:389-400.
- Bishop, S.H., Greenwalt, D.E., Burcham, J.M. (1981). Amino acid cycling in ribbed mussel tissues subjected to hyperosmotic shock. J. Exp. Zool. 215:277-287.
- Bishop, S.H., Ellis, L.L., Burcham, J.M. (1983). Amino acid metabolism in Molluscs. In: The Mollusca, Vol. 1. Metabolic biochemistry and molecular biomechanics, Academic Press. p. 243-327.
- Brussard, P.F. (1974). Population size and natural selection in the land snail Cepaea nemoralis. Nature 251:713-715.
- Buroker, N.E. (1983). Population genetics of the American oyster Crassostrea virginica along the Atlantic coast and the Gulf of Mexico. Mar. Biol. 75:99-112.
- Burton, R.S. (1983). Protein polymorphisms and genetic differentiation of marine invertebrate populations. Mar. Biol. Lett., 4:193-206.
- Burton, R.S., Feldman, M.W. (1983). Physiological effects of an allozyme polymorphism: Glutamate-Pyruvate transaminase and response to hyperosmotic stress in the Copepod Tigriopus californicus. Biochem. Genet. 21:239-251.

- Butler, P.A. (1954). The southern oyster drill. Proc. Nat. Shellfish. Assoc. 44:67-75.
- Byers, B.A. (1983). Enzyme polymorphism associated with habitat choice in the intertidal snail Tegula funebris. Beh. Genet. 13:65-75.
- Clench, W.J. (1947). The genera Purpura and Thais in the western Atlantic. Johnsonia 2:61-91.
- Cole, T.J. (1979). Ecological-genetic studies utilizing the intertidal prosobranch Urosalpinx cinerea (Say) (Gastropoda: Muricidae). Dissertation of Department of Biology, Boston University. 115pp.
- Cooley, N.R. (1978). An inventory of the estuarine fauna in the vicinity of Pensacola, Florida. Florida Marine Research Publications no. 31. Florida Department of Natural Resources Marine Research Laboratory. 119pp.
- Deaton, L.E., Hilbish, T.J., Koehn, R.K. (1984). Protein as a source of amino nitrogen during hyperosmotic volume regulation in the mussel Mytilus edulis. Physiol. Zool. 57:609-619.
- Demoran, W.J. (1988). Monthly oyster reef monitoring and assessment report. Fisheries Management Section, Gulf Coast Research Laboratory, Ocean Springs, MS.
- Findley, A.M., Belisle, B.W., Stickle, W.B. (1978). Effects of salinity fluctuations on the respiration rate of the southern oyster drill Thais haemastoma and the blue crab Callinectes sapidus. Mar. Biol. 49:59-67.
- Finney, D.J. (1971). Probit analysis 3rd. ed. Cambridge Univ. Press, United Kingdom. 333pp.
- Garton, D.W., Stickle, W.B. (1985). Relationship between multiple locus heterozygosity and fitness in the gastropods Thais haemastoma and Thais lamellosa. In: Marine biology of polar regions and effects of stress on marine organisms. eds. J.S. Gray and M.E. Christiansen, John Wiley and Sons Ltd.
- Garthwaite, R. (1986). The genetics of California populations of

- Geukensia demissa (Dillwyn) (Mollusca): Further evidence on the selective importance of leucine aminopeptidase variation in salinity acclimation. Biol. J. Linn. Soc., 28:343-358.
- Grant, W.S., Utter, F.M. (1988). Genetic heterogeneity on different geographic scales in Nucella lamellosa (Prosobranchia, Thaididae). Malacologia 28:275-287.
- Gartner-Kepkay, K.E., Zouros, E. (1983). Genetic differentiation in the face of gene flow: a study of mussel populations from a single Nova Scotian embayment. Can. J. Fish. Aquat. Sci. 40:443-451.
- Garton, D.W. (1984). Relationship between multiple locus heterozygosity and physiological energetics of growth in the estuarine gastropod Thais haemastoma. Physiol. Zool. 57:530-543.
- Garton, D.W., Berg, D.J. (1989). Genetic variation at the Lap locus and ammonia excretion following salinity transfer in an estuarine snail. Comp. Biochem. Physiol., 92A:71-74.
- Garton, D.W., Stickle, W.B. (1980). Effects of temperature and salinity on the predation rate of Thais haemastoma upon Crassostrea virginica. Biol. Bull. 158:48-57.
- Gunter, G. (1979). Studies of the southern oyster borer, Thais haemastoma. Gulf Res. Rept. 6:249-260.
- Hedgpeth, J.W. (1967). Ecological aspects of the Laguna Madre, a hypersaline estuary. In: Estuaries. ed. G.H. Lauff. W.K. Kellogg Biological Station, Michigan State University Publication no. 83. American Association for the Advancement of Science, Washington, D.C.
- Hilbish, T.J., Koehn, R.K. (1985). Dominance in physiological phenotypes and fitness at an enzyme locus. Science, 229:52-54.
- Hilbish, T.J., Deaton, L.E., Koehn, R.K. (1982). Effect of an allozyme polymorphism on regulation of cell volume. Nature, 298:688-689.
- Hildreth, J.E., Stickle, W.B. (1980). The effects of temperature and salinity on the osmotic composition of the southern oyster drill,

- Thais haemastoma. Biol. Bull. 159:148-161.
- Hoffmann, R.J. (1983). Temperature modulation of the kinetics of phosphoglucose isomerase genetic variants from the sea anemone Metridium senile. J. Exp. Zool. 227:361-370.
- Kapper, M.A., Stickle, W.B., Blakeney, E. (1985). Volume regulation and nitrogen metabolism in the Muricid gastropod Thais haemastoma. Biol. Bull. 169:458-475.
- Koehn, R.K., Hall, J.G., Zera, A.J. (1980a). Parallel variation of genotype-dependent aminopeptidase-I activity between Mytilus edulis and Mercenaria mercenaria. Mar. Biol. Lett., 1:245-253.
- Koehn, R.K., Newell, R.I.E., Immermann, F. (1980b). Maintenance of an aminopeptidase allele frequency cline by natural selection. Proc. Nat. Acad. Sci. USA 77:5385-5389.
- Koehn, R.K., Hilbish, T.J. (1987). The adaptive importance of genetic variation. Am. Sci. 75:134-141.
- Kool, S.P. (1987). Significance of radular characters in reconstruction of Thaidid phylogeny (Neogastropoda: Muricacea). The Nautilus 101:117-132.
- Lavie, B., Nevo, E. (1982). Heavy metal selection of phosphoglucose isomerase allozymes in marine gastropods. Mar. Biol. 71:17-22.
- Livingstone, D.R., De Zwaan, A., Leopold, M., Marteion, E. (1983). Studies on the phylogenetic distribution of pyruvate oxidoreductases. Biochem. System. Ecol. 11:415-425.
- McDonald, J.H., Siebenaller, J.F. (1989). Similar geographic variation at the Lap locus in the mussels Mytilus trossulus and M. edulis. Evolution 43:228-231.
- Moore, M.N., Koehn, R.K., Bayne, B.L. (1980). Leucine aminopeptidase (aminopeptidase-I), N-acetyl- $\beta$ -hexosaminidase and lysosomes in the mussel, Mytilus edulis L., in response to salinity changes. J. Exp. Zool. 214:239-249.
- Nevo, E., Shimony, T., Libni, M. (1977). Thermal selection of allozyme

- polymorphisms in barnacles. *Nature*, Lond. 267:699-701.
- Pierce, S.K. (1982). Invertebrate cell volume control mechanisms: A coordinated use of intracellular amino acids and inorganic ions as osmotic solute. *Biol. Bull.* 163:405-419.
- Rose, R.L. (1984). Genetic variation in the oyster, Crassostrea virginica (Gmelin), in relation to environmental variation. *Estuaries* 7:128-132.
- SAS Institute, Inc. (1982). SAS user's guide:statistics. 1982 ed. SAS Institute, Inc., North Carolina.
- Schechter, V. (1943). Tolerance of the snail Thais floridana to waters of low salinity and the effect of size. *Ecology* 24:493-499.
- Shumway, S.E., Gabbott, P.A. (1977). The effect of fluctuating salinity on the concentrations of free amino acids and ninhydrin-positive substances in the adductor muscles of eight species of bivalve molluscs. *J. Exp. Mar. Biol. Ecol.* 29:131-150.
- St. Amant, L.S. (1956). The southern oyster drill. *La. Wild. Comm. Seventh Biannual Rpt.* p. 81-85, 1956-1957.
- Stickle, W.B. (1985). Effects of environmental factor gradients on scope for growth in several species of carnivorous marine invertebrates. from *Marine biology of polar regions and effects of stress on marine organisms.* ed. by J.S. Gray, M.E. Christianson. John Wiley & Sons Ltd. p. 601-616.
- Stickle, W.B., Howey, T.W. (1975). Effects of tidal fluctuations of salinity on hemolymph composition of the southern oyster drill Thais haemastoma. *Mar. Biol.* 33:309-322.
- Watt, W.B. (1983). Adaptation at specific loci, II. demographic and biochemical elements in the maintenance of the colias Pgi polymorphism. *Genetics* 103:691-724.
- Weir, B.S., Cockerham, C.C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution* 38:1358-1370.
- Wickes, M.A., Morgan II, R.P. (1976). Effects of salinity on three



enzymes involved in amino acid metabolism from the American oyster, Crassostrea virginica. Comp. Biochem. Physiol. 53B:339-343.

Widdows, J. (1978a). Combined effects of body size, food concentration and season on the physiology of Mytilus edulis. J. Mar. Biol. Assoc. U.K. 58:109-124.

Widdows, J. (1978b). Physiological indices of stress in Mytilus edulis. J. Mar. Biol. Assoc. U.K. 58:125-142.

Wright, S. (1978). Evolution and the Genetics of populations. Vol. 4. Variability within and among natural populations. University of Chicago, Chicago. 580pp.

Zurburg, W., de Zwaan, A. (1981). The role of amino acids in anaerobiosis and osmoregulation in bivalves. J. Exp. Zool. 215:315-325.

Appendix 2.1 Allelic frequencies of the southern oyster drills, Stramonita canaliculata and S. haemastoma floridana. (RM: relative allelic mobility; N: sample size; Ho: observed heterozygosity; He: expected heterozygosity; CP: Caminada Pass, LA; HP: Henderson Point, MS; MA: Manila, LA; PB: Pensacola Bay, FL; RF: Red Fish Bay, TX; SI: Ship Island, MS; ML: Marineland, FL; SP: South Padre Island, TX; PI: Ponce Inlet, FL)

Locus	Allele (RM)	<u>Stramonita canaliculata</u>						<u>S. h. floridana</u>		
		CP	HP	MA	PB	RF	SI	ML	SP	PI
<u>Adk</u>	N	161	90	88	113	166	90	82	71	19
	120	0.003	0.011	0	0	0.006	0	0	0.007	0.026
	100	0.997	0.983	0.994	1.000	0.991	1.000	0.843	0.722	0.605
	79	0	0.006	0.006	0	0.003	0	0.157	0.271	0.316
	43	0	0	0	0	0	0	0	0	0.053
	Ho	0.006	0.033	0.011	0	0.018	0	0.145	0.333	0.578
	He	0.006	0.033	0.011	0	0.018	0	0.266	0.407	0.545
<u>Aladh</u>	N	179	90	88	120	170	90	112	71	19
	167	0	0	0	0	0	0.006	0	0	0
	153	0.017	0.033	0.028	0.017	0.018	0.017	0	0	0
	133	0.025	0.011	0.006	0.029	0.038	0.011	0.027	0	0
	117	0	0	0	0	0	0	0.004	0	0
	100	0.911	0.939	0.955	0.938	0.918	0.950	0.964	0.993	0.974
	87	0.003	0	0	0	0	0	0	0.007	0.026
	73	0.045	0.017	0.011	0.017	0.026	0.017	0.004	0	0
	Ho	0.162	0.100	0.091	0.125	0.159	0.100	0.071	0.014	0.053
	He	0.168	0.118	0.088	0.120	0.156	0.097	0.070	0.014	0.053
<u>Est1</u>	N	179	90	88	120	170	90	112	71	19
	122	0.003	0	0	0.008	0	0	0.018	0.007	0
	100	0.997	1.000	1.000	0.992	1.000	1.000	0.973	0.993	1.000
	89	0	0	0	0	0	0	0.009	0	0
	Ho	0.006	0	0	0.017	0	0	0.054	0.014	0
	He	0.004	0	0	0.008	0	0	0.053	0.014	0
<u>Est2</u>	N	179	90	88	120	170	90	112	71	19
	107	0	0	0	0	0	0.006	0	0	0
	100	1.000	1.000	1.000	1.000	1.000	0.994	1.000	1.000	1.000
	Ho	0	0	0	0	0	0.011	0	0	0
	He	0	0	0	0	0	0.011	0	0	0
<u>Fum</u>	N	179	90	88	120	170	90	112	71	19
	100	1.000	1.000	1.000	1.000	1.000	1.000	0	0	0
	85	0	0	0	0	0	0	1.000	1.000	1.000
	Ho	0	0	0	0	0	0	0	0	0
	He	0	0	0	0	0	0	0	0	0
<u>Gdh</u>	N	117	90	88	90	156	90	112	71	19
	122	0.004	0	0	0	0	0	0	0	0.105
	100	0.996	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.895

	Ho	0.009	0	0	0	0	0	0	0	0.105
	He	0.009	0	0	0	0	0	0	0	0.193
<u>Got1</u>	N	179	90	88	120	170	90	0	0	0
	125	0.003	0	0	0.004	0	0.011	0	0	0
	100	0.992	1.000	1.000	0.996	1.000	0.989	0	0	0
	50	0.005	0	0	0	0	0	0	0	0
	Ho	0.017	0	0	0.008	0	0.022	0	0	0
	He	0.017	0	0	0.008	0	0.022	0	0	0
<u>Got2</u>	N	179	89	88	120	170	90	112	71	19
	120	0.006	0.006	0	0.004	0.009	0.022	0.004	0	0
	100	0.994	0.994	1.000	0.996	0.991	0.978	0.996	1.000	1.000
	Ho	0.011	0.011	0	0.008	0.018	0.044	0.009	0	0
	He	0.011	0.011	0	0.008	0.018	0.044	0.009	0	0
<u>Gpt1</u>	N	179	90	88	120	170	90	112	71	19
	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	Ho	0	0	0	0	0	0	0	0	0
<u>Gpt2</u>	N	179	90	88	116	169	90	112	71	19
	125	0	0	0	0	0	0	0	0.014	0
	108	0.008	0.011	0.023	0.038	0.041	0.028	0.009	0	0
	100	0.986	0.989	0.977	0.961	0.956	0.967	0.991	0.931	0.921
	83	0.006	0	0	0	0.003	0.005	0	0.055	0.079
	Ho	0.028	0.022	0.045	0.078	0.089	0.067	0.018	0.149	0.158
<u>Lap1</u>	He	0.028	0.022	0.045	0.075	0.085	0.065	0.018	0.132	0.149
	N	72	90	88	113	70	90	112	71	19
	162	0	0	0	0	0	0.006	0	0.014	0
	150	0.007	0.033	0.017	0.035	0.014	0.033	0.058	0.049	0.132
	131	0.020	0.017	0.006	0.013	0.014	0.011	0.009	0	0.026
	125	0.293	0.289	0.358	0.341	0.279	0.294	0.571	0.604	0.526
	110	0.013	0.017	0.006	0.040	0.021	0.017	0.004	0.014	0.026
	100	0.420	0.367	0.381	0.394	0.429	0.428	0.058	0.076	0.053
	90	0.047	0.022	0.011	0.022	0.028	0.017	0.076	0.111	0.079
	84	0.133	0.167	0.188	0.115	0.157	0.156	0.205	0.132	0.158
	56	0.047	0.061	0.028	0.031	0.036	0.033	0.018	0	0
	42	0.020	0.028	0.006	0.009	0.021	0.006	0	0	0
	Ho	0.720	0.700	0.716	0.699	0.657	0.700	0.580	0.486	0.632
	He	0.719	0.752	0.694	0.714	0.716	0.707	0.621	0.601	0.688
<u>Lap2</u>	N	69	0	0	0	69	0	88	71	19
	121	0	0	0	0	0	0	0.006	0.007	0
	108	0.007	0	0	0	0.029	0	0.028	0.042	0.053
	100	0.355	0	0	0	0.290	0	0.943	0.924	0.921
	89	0.565	0	0	0	0.587	0	0.023	0.028	0.026
	79	0.065	0	0	0	0.094	0	0	0	0
	68	0.007	0	0	0	0	0	0	0	0
	Ho	0.609	0	0	0	0.594	0	0.091	0.153	0.158
	He	0.554	0	0	0	0.566	0	0.110	0.145	0.152
<u>LeuAla</u>	N	179	90	88	120	170	90	112	71	19
	117	0.011	0.006	0	0.012	0.009	0.006	0	0.014	0
	111	0	0	0	0	0.003	0	0	0	0
	100	0.978	0.983	1.000	0.975	0.965	0.989	0.009	0.014	0.026



CHAPTER THREE:  
NORMOXIC AND ANOXIC ENERGY METABOLISM OF THE SOUTHERN OYSTER DRILL  
STRAMONITA CANALICULATA DURING SALINITY ACCLIMATION. A DIRECT  
CALORIMETRIC STUDY

ABSTRACT

The energetic cost associated with salinity acclimation was determined in the marine gastropod Stramonita canaliculata by direct calorimetry under normoxic and anoxic conditions. Snails were collected from Caminada Pass near Grand Isle, Louisiana (Longitude 90° 2' W; Latitude 29° 2' N) in September 1987. Metabolic heat flux of snails acclimated to and measured at 10 or 30 ‰ S was similar at 15.06 or 16.39 J g<sup>-1</sup> dry wt h<sup>-1</sup>, respectively, (corresponding to 0.76 or 0.83 ml O<sub>2</sub> g<sup>-1</sup> dry flesh wt h<sup>-1</sup>) under normoxic conditions, and 2.39 or 2.53 J g<sup>-1</sup> dry wt h<sup>-1</sup> under anoxic conditions. Inter-individual variability was high, obscuring the effect of salinity gradient on heat flux. When standardized to the pre-transfer control level of each individual under anoxic conditions, a significant increase (55%) of energy expenditure was observed for snails transferred to hyperosmotic conditions. In contrast, heat flux varied insignificantly in individuals in the anoxic 30 to 10 ‰ S transfer. After transfer of individuals from 10 to 30 ‰ S under normoxic conditions, heat flux was depressed initially to 38% of the control rate, but recovered after 14 h to a higher metabolic rate (56%) than the pre-transfer control rate. After transfer of individuals from 30 to 10 ‰ S under normoxic conditions, the standardized heat flux decreased to 28% of the control rate, followed by a 20 h period of recovery to the control rate. The energy cost of intracellular hypoosmotic regulation was less than hyperosmotic regulation under anoxic conditions. The retraction of the foot of S. canaliculata after normoxic salinity transfers did not generally correlate with the time course of metabolic heat flux.

INTRODUCTION

No single hypothesis can account for the effects of salinity on the metabolic energy demand of marine invertebrates (Kinne 1971). Although

anisosmotic processes are passive in osmoconformers, isosmotic intracellular osmoregulation is an active process. In osmoconforming molluscs, the oxygen consumption rate of Modiolus demissus demissus (Baginski and Pierce 1975), Rangia cuneata (Henry et al. 1980), and Mytilus edulis (Widdows 1985) decreases upon transfer of individuals to hyperosmotic conditions. The oxygen consumption rate also shows a decrease during transfer of individuals to hypoosmotic conditions in Crassostrea virginica, Mercenaria mercenaria, Modiolus demissus (van Winkle 1968), R. cuneata (Henry and Mangum 1980), and M. edulis (van Winkle 1968, Bayne 1973, Widdows 1985). In addition, the oxygen consumption of M. demissus, M. edulis and Stramonita canaliculata declines as the salinity fluctuates in either direction from the acclimation salinity and increases as the ambient salinity returns to the acclimation salinity (Findley et al. 1978, Shumway and Youngson 1979, Stickle and Sabourin 1979). An energetic interpretation of these changes of oxygen consumption is complex, since osmoregulatory energy demand and oxygen availability are involved at the same time in bivalves and snails due to their shell closing and retraction behavior (Bayne 1973).

In previous studies, metabolic rates were measured respirometrically as oxygen consumption. By this method, anaerobic metabolism involved in intracellular osmoregulation (Baginski and Pierce 1975) is not detected. The use of perfusion microcalorimetry allows the determination of total metabolic heat flux, including both aerobic and anaerobic sources of heat (Gnaiger 1983 a). Applying direct calorimetry in this study, we determined (1) the metabolic costs of isosmotic, intracellular hyperosmotic and hypoosmotic regulation of Stramonita canaliculata during salinity transfers of individuals under normoxic and anoxic conditions, and (2) the retraction behavior of S. canaliculata during salinity transfers.

## MATERIALS AND METHODS

Snails

Stramonita canaliculata, ranging in length from 15 to 25mm, were collected from pilings and bulkheads in the vicinity of Caminada Pass near Grand Isle, Louisiana (Longitude 90° 2' W; Latitude 29° 2' N) in September 1987. The ambient water temperature and salinity was 28±1°C and 26 ‰, respectively. The snails were held in 38-liter aquaria (25±0.5°C) containing artificial seawater of the same salinity as in the field. The snails were acclimated by step-wise changes of 2 ‰ S per day to the target salinity and maintained for two weeks before the experiments began. Prior to the experiments, they were provided access to oysters (Crassostrea virginica) ad libitum. Thereafter, the snails were starved for three days before they were used in the experiments.

Calorimetric experiments

In both low to high (10 to 30 ‰) and high to low (30 to 10 ‰) salinity transfers, the heat flux of individual snails was measured in either an open-flow (perfusion) or a static modular microcalorimetry system (model 2277 Thermal Activity Monitor, ThermoMetric) described by Suurkuusk and Wadsö (1982, see also Gnaiger 1983 b). The flow, through the 3.5 ml stainless steel perfusion chamber was 20 ml h<sup>-1</sup> of normoxic (> 150 torr) or anoxic seawater. The level of anoxia (<0.5% air saturation) obtained in the perfusion calorimeter was previously checked with a Cyclobios Twin-Flow respirometer (Gnaiger 1983 c).

Heat flux of individual snails was recorded continuously during perfusion with normoxic seawater for 120 h (24 h: pre-transfer salinity, 25 to 120 h: target salinity). Subsequently, the perfusion medium was switched to seawater equilibrated with nitrogen, and the anoxic heat flux was recorded in several experiments. In the static 25 ml stainless steel chambers, heat flux was recorded in anoxic seawater at the pre-transfer salinity for 24 h and thereafter at the target salinity for another 24 h. Ammonium production under anoxic conditions at both the

pre-transfer and post-transfer salinity was low, ranging from 0.1 to 0.4  $\mu\text{M}$   $\text{NH}_4$  produced per snail over a 24 h period. The  $\text{NH}_4$  concentration at the end of the 24 h experiment would have been no higher than 17  $\mu\text{M}$  in the static chambers while Stramonita canaliculata has been shown to tolerate  $\text{NH}_4$  loading up to 350  $\mu\text{M}$  without any apparent adverse effect (Kapper et al. 1985). The salinity transfer without intrusion of oxygen was possible by flushing anoxic seawater through stainless steel capillaries which were completely sealed during heat flux measurements (Gnaiger 1983 b).

Hourly averages of heat flux were calculated from instantaneous rates read at intervals of 1 min from the chart-recorder traces, expressed in  $\text{J g}^{-1}$  dry flesh  $\text{h}^{-1}$  ( $1 \text{ J h}^{-1} = 0.278 \text{ mW}$ ). The mean pre-transfer steady state flux (control period) was determined as the last 12 h interval at the pre-transfer salinity. The standardized heat flux was calculated as a percentage of this control. One-way ANOVA and Scheffé's multiple comparison tests (SAS Institute, Inc. 1982) were used to test the significance of variation of the standardized mean heat flux.

#### Behavioral observations

The retraction behavior of a separate group of snails was observed in two 38-liter aquaria for 96 h after the target salinity was reached. Separate groups of snails were used for heat flux and behavioral observations because both the perfusion and static calorimeter chambers were opaque and positioned in the thermopiles of the calorimeter, making behavioral observations impossible. The salinity changes were established by gradually siphoning and refilling the aquaria with seawater. Observations were made at 3 h intervals during the first 12 h and thereafter at 12 h intervals. In this study, retraction behavior is calculated as the percentage of snails with the operculum closed and the foot, siphon and tentacles withdrawn.



## RESULTS

The heat flux of Stramonita canaliculata stabilized within the first 12 h period of normoxia after introduction to the pre-transfer salinity. The average heat flux was observed in snails acclimated to and measured at 10 or 30 ‰ S (15.06 or 16.39 J g<sup>-1</sup> dry wt h<sup>-1</sup>, respectively; Table 3.1). Under these conditions, the ratio of calorimetric and simultaneous respirometric measurements were in agreement with the theoretically expected oxycaloric equivalent of 440 to 470 KJ/mol O<sub>2</sub> (Gnaiger 1983 d). This was particularly true when heat and oxygen flux were averaged over several hours (unpublished results). Thus, the average heat flux of 15.06 or 16.39 J g<sup>-1</sup> dry wt h<sup>-1</sup> corresponds to an oxygen flux of 0.76 or 0.83 ml g<sup>-1</sup> dry wt h<sup>-1</sup>.

The heat flux among individuals varied considerably in all experiments (Figs. 3.1 C, 3.2 C, 3.3 B, 3.4 B). The inter-individual variability of metabolic activity obscured, to a large extent, the effects of salinity on heat flux (Table 3.1). Significant salinity effects were readily apparent when the heat flux of an individual during treatments was expressed relative to the pre-transfer period (standardized heat flux expressed as a percentage), because snails with a relatively high metabolic heat flux during the pre-transfer control period had a relatively high flux throughout the experiment.

After transfer from normoxic 10 to 30 ‰ S in the perfusion calorimeter, the mean standardized heat flux decreased within the first 2 h to a minimum of 38% of the pre-transfer control value, and returned to the control level after 14 h (Fig. 3.1 A). In the 30 to 10 ‰ S transfer, the post-transfer standardized heat flux was reduced to a minimum of 28% of the control, followed by a longer period of recovery to the pre-transfer level after 20 h (Fig. 3.2 A).

After the recovery period, standardized heat flux remained significantly higher than the control flux up to 120 h after transfer of snails to hyperosmotic conditions

Table 3.1. *Stramonita canaliculata* mean steady-state heat flux ( $\text{J g}^{-1}$  dry flesh  $\text{h}^{-1}$ ) before and after salinity transfer. Scheffés test for significant variation in standardized mean heat flux is indicated by nonoverlapping capital letters ( $p < 0.05$ ). n= sample size.

Transfer conditions	n	Mean dry wt $\pm$ SE (g)	Time interval (h)	Heat flux $\pm$ SE	Stand. mean heat flux (%)	Scheffés test
10 to 30 ‰ S						
10‰/‰			13 - 24	15.06 $\pm$ 6.08	100	B
30‰/‰ normoxia	6	0.062 $\pm$ 0.032	25 -120	18.20 $\pm$ 5.81	136	B
30‰/‰			25 - 30	5.38 $\pm$ 1.89	38	C
30‰/‰			49 -120	20.87 $\pm$ 6.81	156	A
(30‰/‰ anoxia)	(3)		127 -132	1.15 $\pm$ 0.63	16	C
30 to 10‰ S						
10‰/‰			13 - 24	2.39 $\pm$ 0.39	100	B
30‰/‰ anoxia	5	0.040 $\pm$ 0.013	25 - 48	3.27 $\pm$ 0.51	138	B
30‰/‰			25 - 30	3.66 $\pm$ 0.61	155	A
30‰/‰			37 - 48	2.91 $\pm$ 0.45	122	A,B
30 to 10‰ S						
30‰/‰			13 - 24	16.39 $\pm$ 3.18	100	A
10‰/‰ normoxia	5	0.033 $\pm$ 0.004	25 -120	13.97 $\pm$ 1.54	92	A
10‰/‰			25 - 30	4.32 $\pm$ 0.97	28	B
10‰/‰			49 -120	17.15 $\pm$ 2.14	112	A
(10‰/‰ anoxia)	(1)		127 -132	0.86	10	B
30 to 10‰ S						
30‰/‰			13 - 24	2.53 $\pm$ 0.71	100	A
10‰/‰ anoxia	5	0.159 $\pm$ 0.350	25 - 48	2.09 $\pm$ 0.42	97	A
10‰/‰			25 - 30	2.73 $\pm$ 0.63	125	A
10‰/‰			37 - 48	1.81 $\pm$ 0.34	84	A

Fig. 3.1 Stramonita canaliculata. (A) Standardized heat flux (n=6); ---: means, - - -: SE), and (B) foot retraction (n=9) before and after transfer from 10 to 30 °/ooS under normoxic conditions. (C) Individual traces of heat flux. Horizontal lines indicate time intervals for calculating steady-state heat flux in Table 3.1.

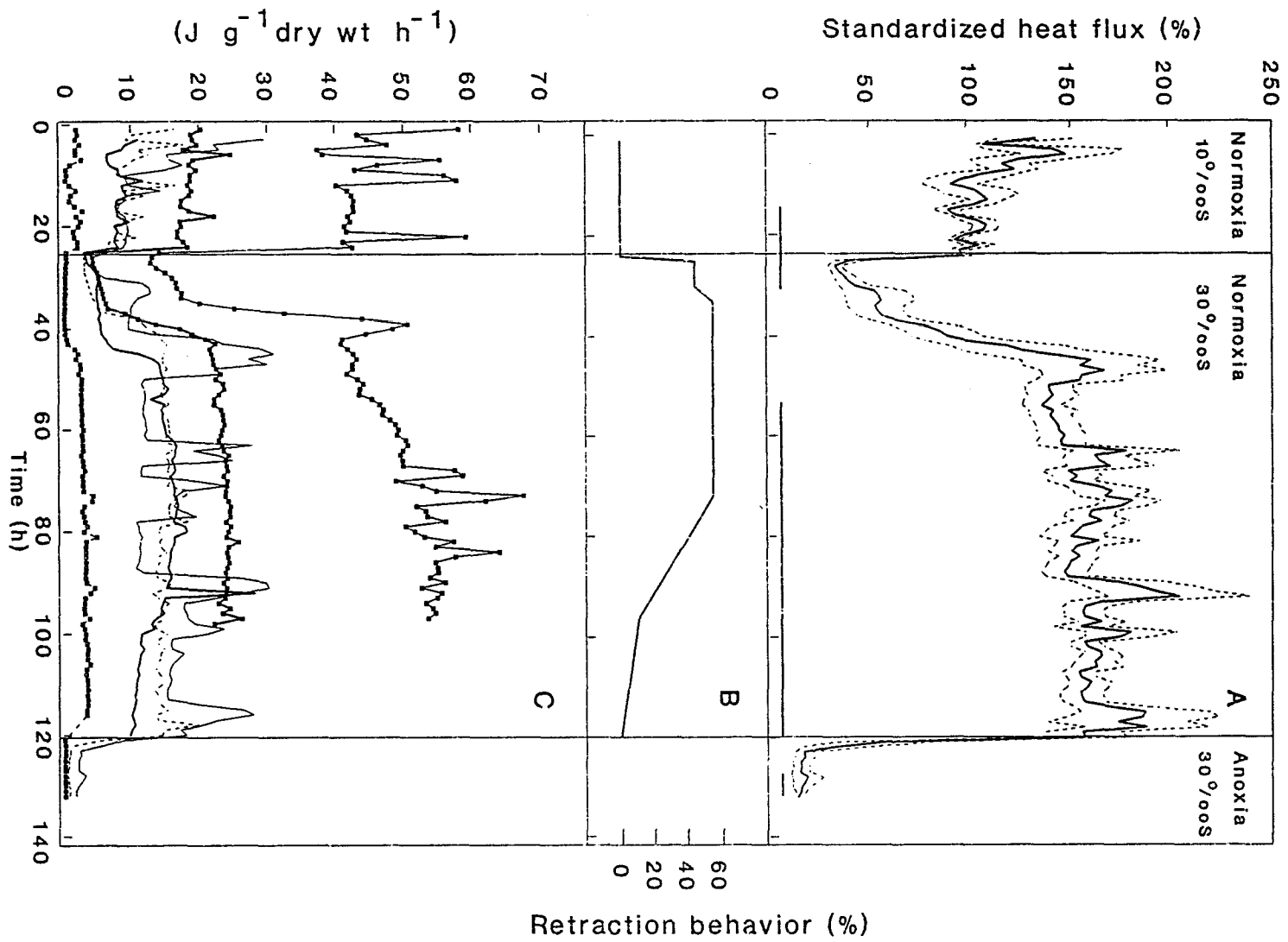


Fig. 3.2 Stramonita canaliculata. (A) Standardized heat flux (n=5); ---: means, - - - : SE), and (B) foot retraction (n=16) before and after transfer from 30 to 10 ‰ S under normoxic conditions. (C) Individual traces of heat flux. Horizontal lines indicate time intervals for calculating steady-state heat flux in Table 3.1.

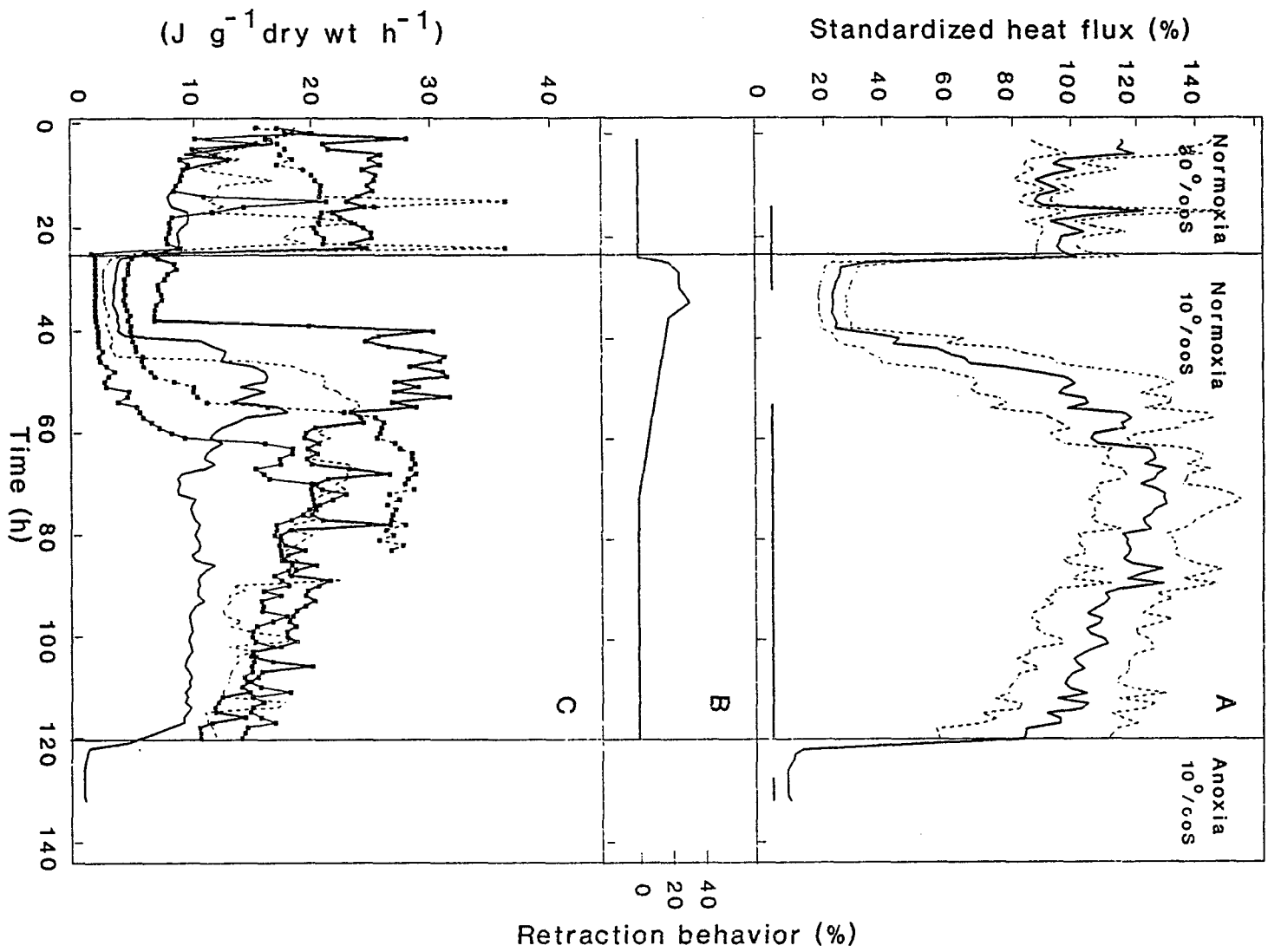


Fig. 3.3 Stramonita canaliculata. (A) Standardized heat flux (n=5; ----  
--: means, - - - : SE), and (B) individual traces of heat flux  
before and after transfer from 10 to 30 ‰ S under anoxic  
conditions. Horizontal lines indicate time intervals for  
calculating steady-state heat flux in Table 3.1.

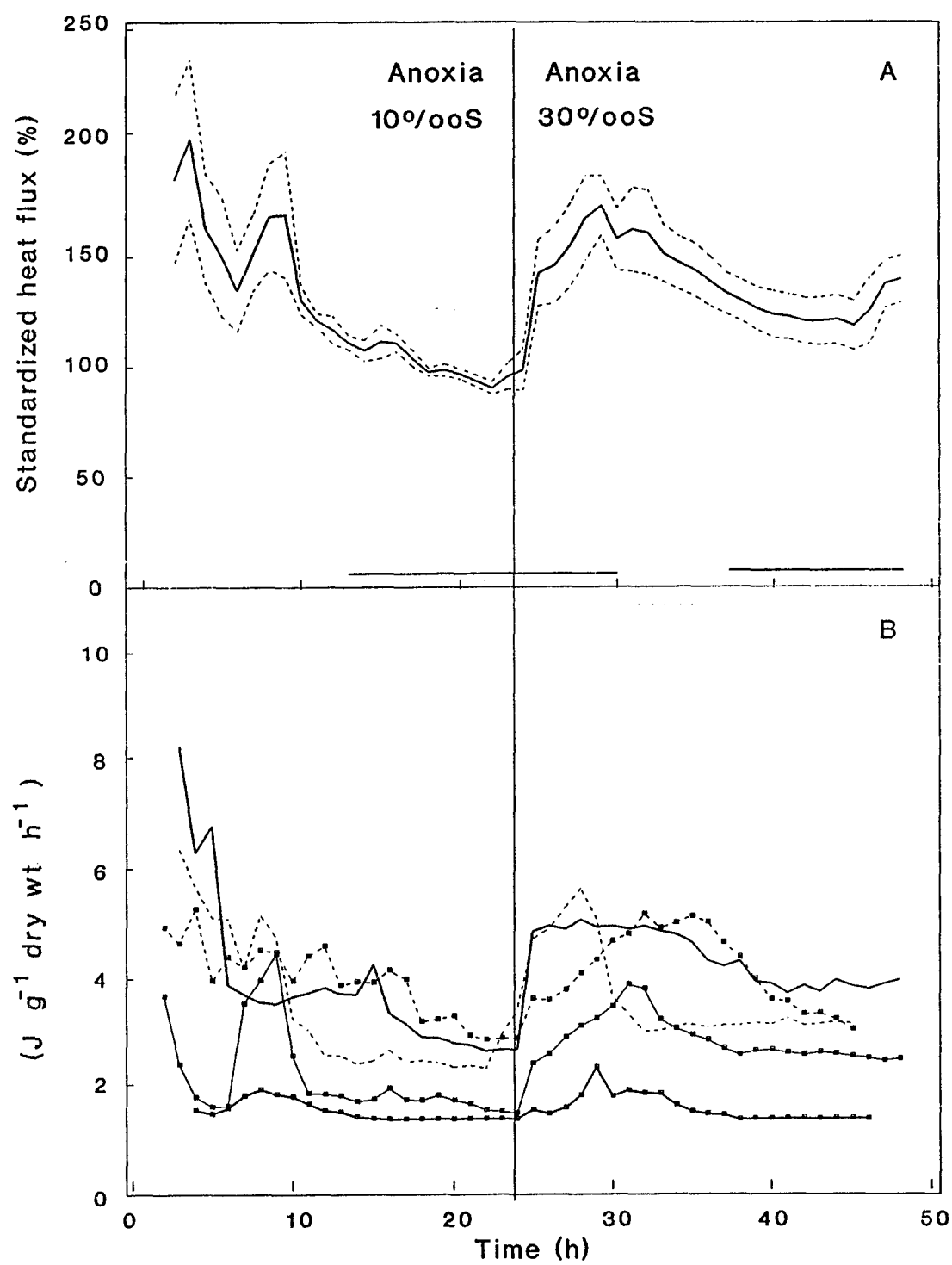
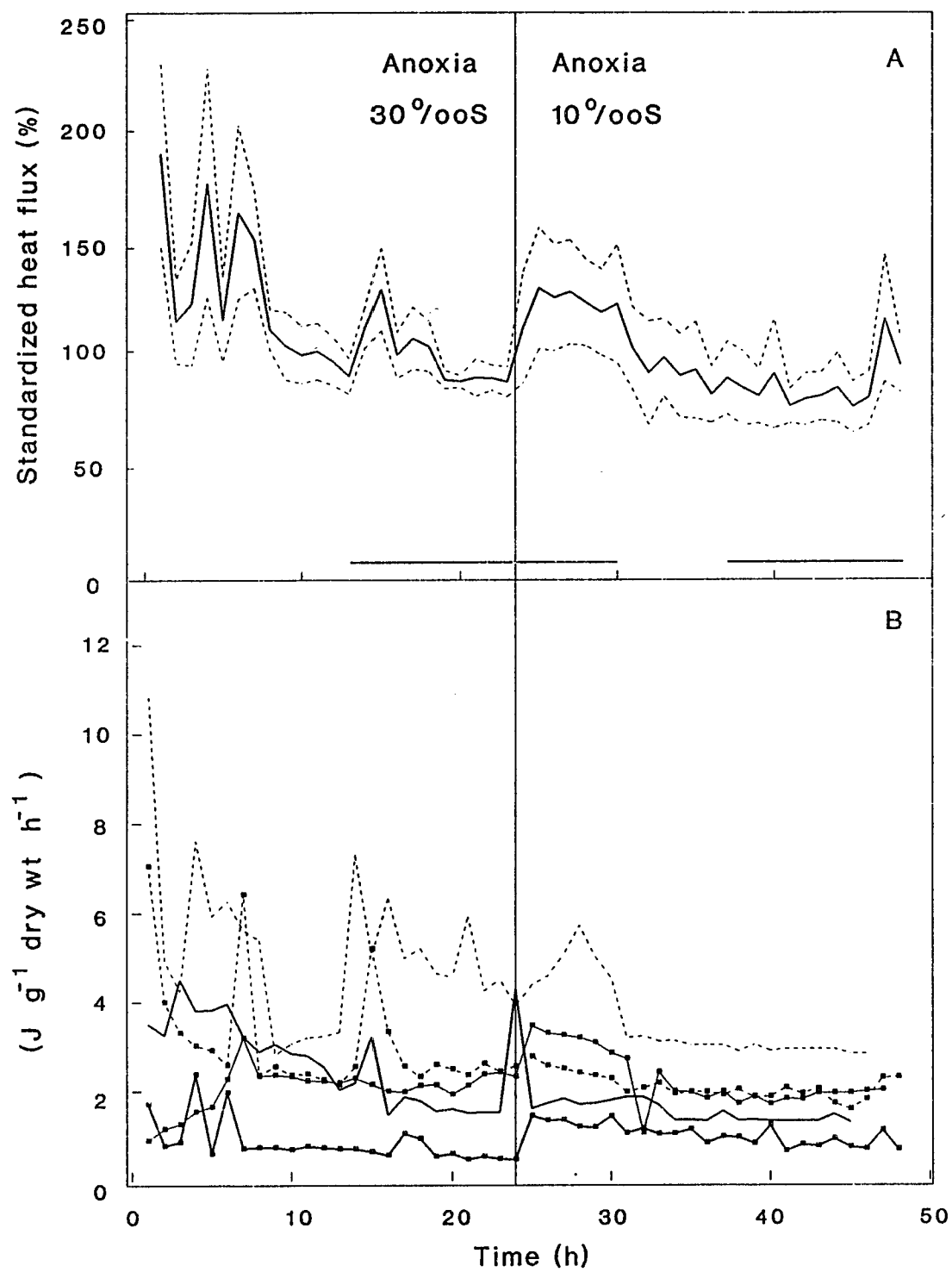




Fig. 3.4 Stramonita canaliculata. (A) Standardized heat flux (n=5; ---- -: means, - - - : SE), and (B) individual traces of heat flux before and after transfer from 30 to 10 ‰ S under anoxic conditions. Horizontal lines indicate time intervals for calculating steady-state heat flux in Table 3.1.



(Fig. 3.1 A). In contrast, the standardized heat flux leveled off at a value insignificantly different from the control after transfer of snails to hypoosmotic conditions (Fig. 3.2 A).

Under anoxic conditions in the closed calorimeter chamber, heat flux of Stramonita canaliculata declined rapidly during the first 12 h and attained a nearly stable level by 24 h. The average anoxic heat flux was similar at 10 and 30 ‰ S, with values of 2.39 and 2.53 J g<sup>-1</sup> dry flesh h<sup>-1</sup>, respectively (Table 3.1). In contrast to the response observed in the normoxic salinity transfers, the standardized heat flux increased shortly after the anoxic hyperosmotic transfer then returned to the control level (Fig. 3.3 A). However, in snails transferred to hypoosmotic conditions, the heat flux did not vary significantly (Fig. 3.4 A). Under long-term anoxia in closed chambers with constant salinity, the heat flux decreased slightly even after 24 h (data not shown), comparable to the results in the final period of the anoxic 30 to 10 ‰ S transfer (Fig. 3.4).

After switching the perfusion system to seawater equilibrated with nitrogen, the metabolic heat flux of a few of the snails declined rapidly to a level below the normoxic level in the preceding normoxic period. However, as measured by Scheffé's multiple comparison test, the anoxic heat flux of 16% in the 10 to 30 ‰ S transfer (3 snails) and 10% in the 30 to 10 ‰ S transfer (1 snail) was not significantly below the minimum normoxic heat flux observed in the transitional phases (Figs. 3.1 A, 3.2 A).

Fifty to sixty percent of the snails had their opercula closed after transfer from normoxic 10 to 30 ‰ S for 2 h. Retraction lasted for about 48 h throughout the period of full recovery of the metabolic heat flux (Fig. 3.1). In the normoxic 30 to 10 ‰ S transition, retraction was less pronounced (33%) than in the transition period of snails from 10 to 30 ‰ S although the heat flux decreased to a larger extent (Fig. 3.2). The snails showed total recovery within 48 h. Thus,

the retraction behavior did not correlate with metabolic heat flux.

#### DISCUSSION

Assessment of the cost of intracellular osmotic regulation under normoxic conditions is complicated by including behavioral responses, particularly in snails which retracted their foot and thus diminish the oxygen supply to their tissues. By setting the environmental oxygen to zero, the complexities of behavioral oxygen limitations are excluded (Shick et al. 1988). Therefore, we applied metabolic calorimetry under anoxic conditions, to quantify directly, for the first time, the energetic cost of osmoregulation in the euryoxic gastropod Stramonita canaliculata. Anoxic heat flux increased by 55% after transfer from 10 to 30 ‰ S (Fig. 3.3 A), indicating that a significant amount of energy is necessary during the early phase of the acclimation of snails to hyperosmotic conditions. In contrast, heat flux varied insignificantly in the anoxic 30 to 10 ‰ S transfer (Fig. 3.4 A). This may indicate that the cost of intracellular isosmotic regulation was undetectably small, or that the oyster drill did not regulate during the first 24 h of transfer to hypoosmotic conditions under anoxia. Further experiments on the time course of intracellular isosmotic regulation are required before either of these possibilities can be ascertained. The undetectable response of heat flux to hypoosmotic conditions cannot be simply due to energy limitation under anoxia, as evidenced by the large anoxic scope in the hyperosmotic transfer to hyperosmotic conditions. The energetic cost of intracellular isosmotic regulation upon transfer of snails to hyperosmotic conditions was higher than that of snails transferred to hypoosmotic conditions, under anoxic conditions (Table 3.1).

Alanine is one of the primary anaerobic end products of Stramonita canaliculata (Kapper and Stickle 1987). Moreover, most of the increase of free amino acid concentration after transfer of S. canaliculata from 10 to 30 ‰ S under normoxic conditions is due to alanine, which

accounts for 66% of the free amino acid pool increase on the first day and for 56% on the second day (Kapper et al. 1985). The time courses of free amino acid accumulation (Kapper et al. 1985) and of depression of metabolic heat flux upon normoxic salinity transfer were similar (Fig. 3.1 A). These results suggest that the initial stages of high-salinity adaptation of the free amino acid pool depend upon the synthesis of amino acids by anaerobic pathways (Baginski and Pierce 1975, 1978). However, it is not known if the metabolic pathways leading to the accumulation of amino acids under normoxic hyperosmotic stress (Bishop et al. 1983) are also employed for anoxic hyperosmotic adaptation.

Generally, the retraction behavior of Stramonita canaliculata did not correlate with metabolic heat flux (Figs. 3.1, 3.2). Similarly, endoscopic observations in the chamber of a calorimeter revealed a poor correspondence between locomotor activities of snails and changes in heat flux (Becker and Lamprecht 1988). It has been suggested that shell closure by Mytilus edulis serves to protect the gill cilia against damage when salinities fluctuate between 33.5 and 0 ‰ (Davenport and Fletcher 1978), but the shell was not used to isolate M. edulis from hypoosmotic seawater if the downward transfer was from 34 to 17 ‰ or above (Costa and Pritchard 1978). Therefore, if the range of salinity transfer is tolerated by the organism, physiological mechanisms in addition to the retraction in S. canaliculata or valve closure in M. edulis must be responsible for variation in metabolic flux. One possible mechanism is volume regulation. In S. canaliculata, however, volume regulation requires 3 d after upward or downward salinity transfers (Kapper et al. 1985), whereas heat flux returns to or above the pre-transfer levels within 14 or 20 h (Figs. 3.1 A, 3.2 A). At the ultrastructural level, stimulation of the lysosomal-vacuolar system in digestive cells as described in M. edulis after transfer from 21 to 35 ‰ for 12 h (Pipe and Moore 1985) may be related to changes in metabolic activity.

The response of Stramonita canaliculata to direct salinity transfer in normoxic seawater shows a similar trend when measured by direct calorimetry (Figs. 3.1, 3.2) or by respirometry (Findley et al. 1978). Heat flux and oxygen consumption decreased less and remained low for a shorter period of time in the low to high salinity transfers than in the high to low salinity transfers. Similarly, the change in oxygen consumption of Mytilus edulis exposed to an increase of salinity from 15 to 30 ‰ was faster than the change to a corresponding decrease (Widdows 1985). On the other hand, the response of the oligohaline clam Rangia cuneata transferred from 2 to 20 ‰ S was slower than the reverse as measured by indirect calorimetry (Henry and Mangum, 1980, Henry et al. 1980).

During the early stage of hyperosmotic regulation, a decrease in oxygen consumption, accompanied by free amino acid accumulation, occurs in the ribbed mussel Modiolus demissus (Baginski and Pierce 1975), Mytilus edulis (Livingstone et al. 1979), Rangia cuneata (Henry et al. 1980), and in the copepod Tigriopus californicus (Goolish and Burton 1989). Under hypoosmotic stress, oxygen consumption increases and free amino-acid levels decrease in T. californicus during the first day after transfer (Goolish and Burton 1989). Similar responses occur in the crustaceans Callinectes sapidus and Eriocheir sinensis (Schoffeniels, 1976 a, b). In contrast, the intracellular free amino acid concentration increases in M. edulis (Livingstone et al. 1979) and Stramonita canaliculata (Kapper et al. 1985) during the first day after transfer from 30 to 15 and 10 ‰ S, respectively, while respiration or heat flux decrease during the first day after transfer (Fig. 3.2 A). The different patterns of oxygen flux in Tigriopus californicus and heat flux in S. canaliculata may indicate different mechanisms for adjusting the free amino acid pool to hypoosmotic stress in gastropods and crustaceans.

The regulation of the free amino-acid pool during salinity

adaptation is, in part, genetically dependent. The rate of regulation of free amino acid pool size and amino acid excretion is correlated with the expression of different LAP (aminopeptidase-I) allozymes in Mytilus edulis (Hilbish et al. 1982, Deaton et al. 1984), as is the rate of ammonia excretion in Stramonita canaliculata (Garton and Berg 1989). The rate of free amino acid pool size change of Tigriopus californicus is also correlated with variation in glutamate-pyruvate transaminase (GPT) allozymes (Burton and Feldman 1983). Therefore, the variation in heat flux among individual snails (Figs. 3.1 C, 3.2 C, 3.3 B, 3.4 B) may be partially related to the different genotypes of key metabolic enzymes involved in free amino-acid pool-regulation.

## ACKNOWLEDGEMENTS

We would particularly like to acknowledge LKB-ThermoMetric, Stockholm for provision of a Thermal Activity Monitor model 2277 microcalorimeter. We thank Dr. W. G. Henk of the LSU Veterinary School for the computer facilities, and among many others, Dr. S. Bishop for his helpful comments. E. Gnaiger was supported by a Schrödinger fellowship from the Austrian FWF and the Visiting Investigator Program at LSU.



## LITERATURE CITED

- Baginski, R. M., Pierce, S. K. Jr. (1975). Anaerobiosis: a possible source of osmotic solute for high-salinity acclimation in marine molluscs. *J. Exp. Zool.* 62:589-598.
- Baginski, R. M., Pierce, S.K. Jr. (1978). A comparison of amino acid accumulation during high salinity adaptation with anaerobic metabolism in the ribbed mussel, Modiolus demissus demissus. *J. Exp. Zool.* 203:419-428.
- Bayne, B. (1973). The responses of three species of bivalve mollusc to declining oxygen tension at reduced salinity. *Comp. Biochem. Physiol.* 45A:793-806.
- Becker, W., Lamprecht, I. (1988). Endoscopic observations of locomotor activities during calorimetric experiments. In: Gnaiger, E. (ed.) *Physiological and biochemical microcalorimetry*, Veröffentl. Univ. Innsbruck, 168:43-44.
- Bishop, S. H., Ellis, L. L., Burcham, J. M. (1983). Amino acid metabolism in mollusks. In: The Mollusca, Vol. I, Academic Press, New York, p. 243-327.
- Burton, R. S., Feldman, M. W. (1983). Physiological effects of an allozyme polymorphism: glutamate-pyruvate transaminase and response to hyperosmotic stress in the copepod Tigriopus californicus. *Biochem. Genet.* 21:239-251.
- Costa, C. J., Pritchard, A. W. (1978). The response of Mytilus edulis to short duration hypoosmotic stress. *Comp. Biochem. Physiol.* 61A:149-155.
- Davenport, J., Fletcher, J. S. (1978). The effects of simulated estuarine mantle cavity conditions upon the activity of the frontal gill cilia of Mytilus edulis. *J. mar. biol. Ass. U.K.* 58:671-681.
- Deaton, L. E., Hilbish, T. J., Koehn, R. K. (1984). Protein as a source of amino nitrogen during hyperosmotic volume regulation in the

- mussel Mytilus edulis. *Physiol. Zool.* 57:609-619.
- Findley, A. M., Belisle, B. W., Stickle, W. B. (1978). Effects of salinity fluctuations on the respiration rate of the southern oyster drill Thais haemastoma and the blue crab Callinectes sapidus. *Mar. Biol.* 49:59-67.
- Garton, D. W., Berg, D. J. (1989). Genetic variation at the LAP locus and ammonia excretion following salinity transfer in an estuarine snail. *Comp. Biochem. Physiol.* 92A:71-74.
- Gnaiger, E. (1983 a). Heat dissipation and energetic efficiency in animal anoxiosis. Economy contra power. *J. Exp. Zool.* 228:471-490.
- Gnaiger, E. (1983 b). Microcalorimetric monitoring of biological activities. Ecological and toxicological studies in aquatic animals. *Science Tools* 30:21-26.
- Gnaiger, E. (1983 c). The twin-flow microrespirometer and simultaneous calorimetry. In: Gnaiger, E., Forstner, H. (ed.) *Polarographic oxygen sensors. aquatic and physiological applications*, Springer, Berlin, Heidelberg, New York, p. 134-166.
- Gnaiger, E. (1983 d). Calculation of energetic and biochemical equivalents of respiratory oxygen consumption. In: Gnaiger, E., Forstner, H. (ed.) *Polarographic oxygen sensors. aquatic and physiological applications*, Springer, Berlin, Heidelberg, New York, p. 337-345.
- Goolish, E., Burton, R. S. (1989). Energetics of osmoregulation in an intertidal copepod: effects of anoxia and lipid reserves on the pattern of free amino acid accumulation. *Funct. Ecol.* 3:81-89.
- Henry, R. P., Mangum, C. P. (1980). Salt and water balance in the oligohaline clam, Rangia cuneata. III. Reduction of the free amino acid pool during low salinity adaptation. *J. Exp. Zool.* 211:25-32.
- Henry, R. P., Mangum C. P., Webb, K. L. (1980). Salt and water balance

- in the oligohaline clam, Rangia cuneata. II. Accumulation of intracellular free amino acids during high salinity adaptation. J. Exp. Zool. 211:11-24.
- Hilbish, T. J., Deaton, L. E., Koehn, R. K. (1982). Effect of an allozyme polymorphism on regulation of cell volume. Nature 298:688-689.
- Kapper, M. A., Stickle, W. B. (1987). Metabolic responses of the estuarine gastropod Thais haemastoma to hypoxia. Physiol. Zool. 60:159-173.
- Kapper, M.A., Stickle, W.B., Blakeney, E. (1985). Volume regulation and nitrogen metabolism in the muricid gastropod Thais haemastoma. Biol. Bull. 169:458-475.
- Kinne, O. (1971). Salinity: Invertebrates. In Kinne, O. (ed.) Marine ecology, Vol. I, Wiley, Chichester, Wiley-Interscience, London, p. 821-995.
- Livingstone, D. R., Widdows, J., Fieth, P. (1979). Aspects of nitrogen metabolism of the common mussel Mytilus edulis: adaptation to abrupt and fluctuating changes in salinity. Mar. Biol. 53:41-55.
- Pipe, R. K., Moore, M. N. (1985). Ultrastructural changes in the lysosomal-vacuolar system in digestive cells of Mytilus edulis as a response to increased salinity. Mar. Biol. 87:157-163.
- SAS Institute, Inc. (1982). SAS user's guide: statistics, 1982 ed. SAS Institute, Inc., Cary, North Carolina.
- Schoffeniels, E. (1976 a). Adaptations with respect to salinity. Biochem. Soc. Symp. 41:179-204.
- Schoffeniels, E. (1976 b). Biochemical approaches to osmoregulatory processes in Crustacea. In: Davies, P. S. (ed.) Perspectives in experimental biology, Vol. I Zoology, Pergamon Press, Oxford, p. 107-124.
- Shick, J. M., Widdows, J., Gnaiger, E. (1988). Calorimetric studies of behavior, metabolism and energetics of sessile intertidal animals.

- Am. Zool. 28:161-181.
- Shumway, S. E., Youngson, A. (1979). The effects of fluctuating salinity on the physiology of Modiolus demissus (Dillwyn). J. Exp. Mar. Biol. Ecol. 40:167-181.
- Stickle, W.B., Sabourin, T. D. (1979). Effects of salinity on the respiration and heart rate of the common mussel, Mytilus edulis L., and the black chiton, Katherina tunicata (Wood). J. Exp. Mar. Biol. Ecol. 41:257-268.
- Suurkuusk, J., Wadsö, I. (1982). A multiple channel modular microcalorimeter. Chimica Scr. 20:155-163.
- Widdows, J. (1985). The effects of fluctuating and abrupt changes in salinity on the performance of Mytilus edulis. In: Gray, J.S., Christiansen, M. E. (ed.) Marine biology of polar regions and effects of stress on marine organisms, John Wiley and Sons, Chichester, p. 555-566.
- van Winkle, W. (1968). The effects of season, temperature and salinity on the oxygen consumption of bivalve gill tissue. Comp. Biochem. Physiol. 26:69-80.

### Summary

Salinity adaptation in the southern oyster drills, Stramonita haemastoma canaliculata and S. h. floridana is primarily due to environmental effects as observed in the salinity tolerance and microcalorimetry studies. Studies on shell morphology, radular morphology and enzyme criteria indicate that allozyme data is more powerful in detecting the systematic relationships of Stramonita haemastoma. Fixed allelic differences between S. h. canaliculata and S. h. floridana are in 4 of the 16 enzyme loci and Nei's genetic distance is 0.28.

Salinity-related phenotypic variation determined as LC-50 values is different for Stramonita canaliculata and S. haemastoma floridana. The 28-day high-salinity LC-50 values are lower in S. canaliculata than in S. h. floridana. Three of the ten salinity-related enzyme loci (i.e. Lap1, Lap2, and LeuAla) are different from each other with respect to the predominant alleles in S. canaliculata and S. h. floridana. The slight phenotypic differences in salinity tolerance between the two species may, in part, be due to their genetic differences. The 28-day low-salinity LC-50 is from 3.5 to 7.1 ‰ S. The general trend for salinity tolerance among populations is that snails from medium to high salinity areas exhibit a higher high-salinity tolerance limit with a shorter period to reach a stable high-salinity tolerance limit than snails from low to medium salinity areas. In contrast, the low-salinity LC-50 values exhibit the opposite trend. Although phenotypic differences in salinity tolerance exist among populations of both species, the salinity-related enzyme loci exhibit little genetic variation among these populations as indicated in the small  $F_{DP}$  values.

The heat dissipation rate is less depressed with a shorter recovery period in the normoxic 10 to 30 ‰ S transfer than in the normoxic 30

to 10 ‰ S transfer. Under anoxia, the energetic cost of intracellular isosmotic regulation upon transfer of snails to hyperosmotic conditions is higher than that of snails transferred to hypoosmotic conditions. Anoxic heat flux increases by 55% after transfer from 10 to 30 ‰ S, indicating that a significant amount of energy is necessary during the early phase of the acclimation of snails to hyperosmotic conditions. In contrast, heat flux varies insignificantly in the anoxic 30 to 10 ‰ S transfer.

The heat flux among individuals varies considerably in all experiments. Snails with a relatively high metabolic heat flux during the pre-transfer control period maintain a relatively high flux throughout the experiment. This indicates the phenotypic variations exist among individuals during salinity adaptation. It has been shown that the adjustment of the free amino acid pool size during salinity adaptation is, in part, genetically dependent. The rate of ammonia excretion has been correlated with Lap genotypes in the mussel Mytilus edulis (Hilbish et al. 1982 and Deaton et al. 1984) and in Stramonita haemastoma (Garton and Berg 1989). The rate of free amino acid pool change in the copepod Tigriopus californicus has also been correlated with variation in Gpt genotypes (Burton and Feldman 1983). Therefore, the variation in heat flux among individual snails may be partially related to the different genotypes of salinity-related enzyme loci which itself is indicated by enzyme polymorphism, e.g. Lap1 and Lap2, among the individuals used in the microcalorimetry studies (unpublished data) or in the genetic studies in the populations of S. canaliculata and S. h. floridana.

The results of this study indicate that much of the phenotypic variation is the result of physiological effects induced by environmental salinity changes rather than genetic variation. However,

considerable evidence from other studies indicates that adaptation to environmental changes in organisms is genetically dependent. Several factors likely account for differences observed in this study and those found earlier. Correlation of allozyme polymorphism in Lap with environmental salinity has been documented in the mussel Mytilus edulis (Koehn et al. 1980a, McDonald and Siebenaller 1989, Gartner-Kepkay and Zouros 1983), the mussel M. trossulus (McDonald and Siebenaller 1989), the bivalve Geukensia demissa (Garthwaite 1986), and the oyster Crassostrea virginica (Buroker 1983). Correlation of Gpt polymorphism with salinity was also reported in the copepod Tigriopus californicus (Burton and Feldman 1983). Because greater rates of nitrogen loss and tissue weight loss are Lap genotype-dependent in Mytilus edulis, the depletion of nitrogen resources results in genotype-dependent mortality (Hilbish and Koehn 1985, Koehn and Hilbish 1987, Moore et al. 1980, Hilbish et al. 1982, Koehn et al. 1980b). Commonly, in the cited studies, the organisms which have been used, were either sessile organisms or herbivores. Therefore, nitrogen resources may be a limiting factor. Oxygen : nitrogen (O:N) ratios indicate that S. canaliculata has a protein-oriented metabolism (Stickle 1985). Oyster drills, including Stramonita canaliculata and S. haemastoma floridana are carnivorous; therefore, protein resources are much more available to them than to filter feeders or algal scrappers.

In addition, when large amplitudes of salinity variation occur, oyster drills may close their operculum and switch to partial anaerobiosis or move to deeper channels with higher salinity seawater (Butler 1954). Consequently, the protein-oriented metabolism and the avoidance behavior to rapid salinity changes in oyster drills may diminish the necessity of genotypic adaptation at salinity-related enzyme loci.

## LITERATURE CITED

- Abbott, R.T. (1974). American seashells- the marine mollusca of the Atlantic and Pacific coasts of North America. Van Nostrand Reinhold Co., New York.
- Andrews, J. (1971). Sea shells of the Texas coast. University of Texas Press, Austin.
- Bishop, S.H., Greenwalt, D.E., Burcham, J.M. (1981). Amino acid cycling in ribbed mussel tissues subjected to hyperosmotic shock. J. Exp. Zool. 215:277-287.
- Bishop, S.H., Ellis, L.L., Burcham, J.M. (1983). Amino acid metabolism in Molluscs. In: The Mollusca, Vol. 1. Metabolic biochemistry and molecular biomechanics, Academic Press. p.243-327.
- Buroker, N.E. (1983). Population genetics of the American oyster Crassostrea virginica along the Atlantic coast and the Gulf of Mexico. Mar. Biol. 75:99-112.
- Burton, R.S., Feldman, M.W. (1983). Physiological effects of an allozyme polymorphism: glutamate-pyruvate transaminase and response to hyperosmotic stress in the copepod Tigriopus californicus. Biochem. Genet. 21:239-251.
- Butler, P.A. (1954). The southern oyster drill. Proc. Natl. Shellfish. Assoc. 44:67-75.
- Clench, W.J. (1947). The genera Purpura and Thais in the western Atlantic. Johnsonia 2:61-91.
- Deaton, L.E., Hilbish, T.J., Koehn, R.K. (1984). Protein as a source of amino nitrogen during hyperosmotic volume regulation in the mussel Mytilus edulis. Physiol. Zool. 57:609-619.
- Findley, A.M., Belisle, B.W., Stickle, W.B. (1978). Effects of salinity fluctuations on the respiration rate of the southern oyster drill Thais haemastoma and the blue crab Callinectes



- sapidus. Mar. Biol. 49:59-67.
- Garthwaite, R. (1986). The genetics of California populations of Geukensia demissa (Dillwyn) (Mollusca): further evidence on the selective importance of leucine aminopeptidase variation in salinity acclimation. Biol. J. Linn. Soc. 28:343-358.
- Garthwaite-Kepkay, K.E., Zouros, E. (1983). Genetic differentiation in the face of gene flow: a study of mussel populations from a single Nova Scotian embayment. Can. J. Fish. Aquat. Sci. 40:443-451.
- Garton, D.W. (1984). Relationship between multiple locus heterozygosity and physiological energetics of growth in the estuarine gastropod Thais haemastoma. Physiol. Zool. 57:530-543.
- Garton, D.W., Stickle, W.B. (1985). Relationship between multiple locus heterozygosity and fitness in the gastropods Thais haemastoma and Thais lamellosa. In: Marine biology of polar regions and effects of stress on marine organisms. eds. J.S. Gray and M.E. Christiansen, John Wiley and Sons Ltd.
- Garton, D.W., Berg, D.J. (1989). Genetic variation at the Lap locus and ammonia excretion following salinity transfer in an estuarine snail. Comp. Biochem. Physiol. 92A:71-74.
- Garton, D.W., Stickle, W.B. (1980). Effects of temperature and salinity on the predation rate of Thais haemastoma upon Crassostrea virginica. Biol. Bull. 158:48-57.
- Garton, D.W., Stickle, W.B. (1985). Relationship between multiple locus heterozygosity and fitness in the gastropods Thais haemastoma and Thais lamellosa. In: Proceedings of the 18th European Marine Biology Symposium, 1983. eds. J.S. Gray and M.E. Christiansen. Wiley and Sons, London. p. 545-553.
- Gunter, R. (1979). Studies of the southern oyster borer, Thais haemastoma. Gulf Res. Rept. 6:249-260.

- Hilbish, T.J., Deaton, L.E., Koehn, R.K. (1982). Effect of an allozyme polymorphism on regulation of cell volume. *Nature* 298:688-689.
- Hilbish, T.J., Koehn, R.K. (1985). Dominance in physiological phenotypes and fitness at an enzyme locus. *Science* 229:52-54.
- Hildreth, J., Stickle, W.B. (1980). Effects of temperature and salinity upon the osmotic composition of the southern oyster drill, Thais haemastoma. *Biol. Bull.* 159:148-161.
- Kapper, M.A., Stickle, W.B., Blakeney, E. (1985). Volume regulation and nitrogen metabolism in the Muricid gastropod Thais haemastoma. *Biol. Bull.* 169:458-475.
- Koehn, R.K., Hall, J.G., Zera, A.J. (1980a). Parallel variation of genotype-dependent aminopeptidase-I activity between Mytilus edulis and Mercenaria mercenaria. *Mar. Biol. Lett.* 1:245-253.
- Koehn, R.K., Newell, R.I.E., Immermann, F. (1980b). Maintenance of an aminopeptidase allele frequency cline by natural selection. *Proc. Natl. Acad. Sci. USA* 77:5385-5389.
- Koehn, R.K., Hilbish, T.J. (1987). The adaptive importance of genetic variation. *Am. Scient.* 75:134-141.
- Kool, S.P. (1987). Significance of radular characters in reconstruction of Thaidid phylogeny (Neogastropoda: Muricacea). *The Nautilus* 101:117-132.
- Kool, S.P. (1989). Phylogenetic analysis of the subfamily Thaidinae (Prosobranchia: Neogastropoda: Muricacea). Dissertation. The George Washington University, Washington, D.C. pp. 342.
- Livingstone, D.R., De Zwaan, A., Leopold, M., Marteijn E. (1983). Studies on the phylogenetic distribution of pyruvate oxidoreductases. *Biochem. System. Ecol.* 11:415-425.
- McDonald, J.H., Siebenaller, J.F. (1989). Similar geographic variation at the Lap locus in the mussels Mytilus trossulus and M. edulis.

- Evol. 43:228-231.
- Moore, D.R. (1961). The marine and brackish water Mollusca of the state of Mississippi. Gulf Res. Rept. 1:1-58.
- Moore, M.N., Koehn, R.K., Bayne, B.L. (1980). Leucine aminopeptidase (aminopeptidase-I), N-acetyl- $\beta$ -hexosaminidase and lysosomes in the mussel, Mytilus edulis L., in response to salinity changes. J. Exp. Zool. 214:239-249.
- Prosser, C.L. (1986). Adaptational biology- molecules to organisms. John Wiley & Sons, New York. p.1-26.
- Stickle, W.B. (1985). Effects of environmental factor gradients on scope for growth in several species of carnivorous marine invertebrates. In: Marine biology of polar regions and effects of stress on marine organisms. ed. J.S. Gray, M.E. Christiansen. John Wiley & Sons Ltd. p. 601-616.
- Stickle, W.B., Howey, T.W. (1975). Effects of tidal fluctuation of salinity on hemolymph composition of the southern oyster drill Thais haemastoma. Mar. Biol. 33:309-322.
- Stickle, W.B., Kapper, M.A., Blakeney, E., Bayne, B.L. (1985). Effects of salinity on the nitrogen metabolism of the muricid gastropod, Thais (=Nucella) lapillus (L.) (Mollusca: Prosobranchia). J. exp. Mar. Biol. Ecol. 91:1-16.
- Walker, R.L. (1982). The gastropod Thais haemastoma in Georgia: T. h. floridana or T. h. canaliculata ? Gulf Res. Rept. 7:183-184.
- Wickes, M.A., Morgan II, R.P. (1976). Effects of salinity on three enzymes involved in amino acid metabolism from the American oyster, Crassostrea virginica. Comp. Biochem. Physiol. 53B:339-343.

COPYRIGHT RELEASE



Mrs. R. Scheuermann  
Springer-Verlag Heidelberg  
Journal Production Department III  
West Germany (FRG)

Mar. 5, 1990

Dear Mrs. Scheuermann:

I would like to request a written permission that the article entitled " Normoxic and anoxic energy-metabolism of the southern oyster drill Thais haemastoma during salinity acclimation. A direct calorimetric study " by L.L. Liu, W.B. Stickle and E. Gnaiger (Marine Biology; MS no. 1/376) can be included as the third chapter of my dissertation.

Thank you very much.

Sincerely yours,

Li-Lian Liu

# PERMISSION GRANTED

Insofar as it concerns original material  
which do not carry references to the source  
of other publications,  
provided that

- author permission is also obtained
- and full credit is given to the original publication

SPRINGER-VERLAG  
HEIDELBERG March 05, 1990

### Vita

Li-Lian Liu was born 31 October 1958 in Taipei, Taiwan. She attended the Chang-hua Girls' High School in Chang-hua, Taiwan. Three years later, she entered National Taiwan Normal University majoring in Biology. In 1981, upon completion of her studies at National Taiwan Normal University, she entered the master's degree program at National Sun Yat-Sen University and in 1983 she received a Master of Science Degree in Marine Biology. She remained there as an instructor from August 1983 through December 1985. She entered the Ph.D. program in the Department of Zoology and Physiology at LSU in January 1986. She was awarded the fellowship of National Science Council of Taiwan in 1986-1988 and Local Sigma XI Graduate Student Grand-In Aid in 1987. Currently, she is a candidate for the Degree of Doctor of Philosophy in Zoology.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Li Lian Liu

Major Field: Zoology

Title of Dissertation: Genotypic and phenotypic adaptations of the southern oyster drills,  
Stramonita canaliculata and S. haemastoma floridana to salinity

Approved:

William B. Stickle

Major Professor and Chairman

F. Glen Hembry

Dean of the Graduate School

EXAMINING COMMITTEE:

Thomas Dietz

Joseph F. Siebenaller

Don G. Brantlett

Ken Brown

John W. Henry

Date of Examination:

February 9, 1990